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A REVIEW OF ADRENAL CORTICAL HYPERTROPHY[1]

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HYPERTROPHY of the adrenal glands has been described in a bewildering variety of circumstances, but few attempts have been made to determine the nature of the humoral or neural stimuli that may initiate the process. It is true that many workers in this field have used such phrases as 'reaction to stress,' 'reaction to unfavorable environment,' 'alarm reaction,' or 'protective power of the adrenals,' but these concepts can be regarded as no more than vaguely descriptive, and therefore legitimate targets for more precise methods of analysis. The phrase 'reaction to stress,' in a general way, describes many of the conditions we propose to discuss, but it gives us no understanding of the possible mechanisms that may stimulate the adrenals to hypertrophy.

Much of the work to be reviewed is difficult to evaluate for many reasons, some of which illustrate the inherent complexity of the problem. For example, there have been few attempts to estimate the extent of cortical hypertrophy in abnormally large glands. Ideally, an effort should be made to determine not only the weight of adrenal cortical tissue but also pertinent data on its water, protein and fat content. In the absence of such determinations, however, one must admit the possibility that an increase in weight of whole glands may represent some degree of increase in cortical cell mass. Even more distressing than a failure to partition cortical and medullary hypertrophy, is the omission of gland weights from published reports. Occasionally we have felt obliged to list as causes of adrenal enlargement certain agents (usually individual toxins) which were mentioned no more than casually by workers who observed their effects.

Other difficulties in interpretation of results arise from inadequately controlled experiments. The well-marked sex difference in adrenal size and the variation in gland weight with the estrous cycle in the female are facts which

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are often ignored. Again, many of the conditions to be described (such as anoxia, exposure to heat, injection with a variety of noxious agents, and traumatic shock) are often associated with a diminution in food intake which, depending on its extent, can cause either a relative increase or decrease in gland weight. Yet, proper inanition controls are reported only rarely.

It is well-recognized at the outset that the words 'hypertrophy' and 'activation' (which can be defined as an increased rate of hormone production) cannot be used interchangeably. In certain instances, such as in the case of compensatory hypertrophy, there appears to be a correlation between the hormone requirement of the organism and the ultimate extent of cortical cell mass, but this correlation cannot be assumed in all other conditions in which there is an increase in the amount of adrenal cortical tissue. However, the fundamental biochemical mechanisms that control the phenomenon of compensatory hypertrophy are very likely identical with those involved in the maintenance of normal adrenal size, and it is highly improbable that a qualitatively different mechanism comes into play when the hormone requirement of the animal is abnormally high.

Other criteria of activation have been sought, but none has been found to be infallible. Depletion of the lipoid content of the adrenals following the administration of anterior pituitary extract was reported by Houssay and his coworkers (1933). Selye (1936, 1937), Donahue and Parkins (1935), and Sarason (1943, *b*) have observed a diminution in the fat content of the glands under various conditions of 'stress.' This histochemical reaction has been widely interpreted as signifying an increased rate of hormone production. However, Bennett (1940) who has had an extensive experience with the histochemical behavior of the adrenal cortex, warns that no conclusions concerning the level of activity of the adrenal glands can be drawn from their histologic appearance.

Physiologic evidences of increased adrenal activity are necessarily indirect, and, therefore, not entirely reliable. Three methods have been used to estimate the rate of hormone production; one is based on the comparison of an experimental animal which is suspected of producing large amounts of hormone with normal animals given cortical extract or a physiologically active steroid; another, used in the study of anoxia, attempts to correlate the findings in the intact animal exposed to a stress, with the cortical hormone requirement of an adrenalectomized animal exposed to the same stress; the third rests upon the estimation of the quantity of cortical hormone excreted in the urine. The first two of these methods can be applied successfully in only a limited number of circumstances, whereas the third (apart from being difficult) is open to the objection that an increase in hormone excretion does not necessarily mean that there is an increase in hormone requirement in any given condition, and, conceivably, it may indicate the reverse. Conversely, there may be increased hormone requirement which results in increased hormone production without an abnormally large amount of the material appearing in the urine.

Increased cortical cell mass, lipoid depletion and even physiologic evi-

dences for increased hormone production are admittedly inconclusive proof of increased adrenal cortical activity. The rigorous application of these criteria to the experimental data under review would have resulted in the exclusion of a large proportion of them. On the other hand, examination of incomplete data in conjunction with other pertinent observations seems to us to be a justifiable procedure.

Throughout this review, then, the experimental evidence has been examined from two points of view. *a*) Since the role of the anterior pituitary in the maintenance of normal adrenal size has been well substantiated, and since there is present in anterior pituitary extracts a more or less specific substance that produces hypertrophy of the adrenal cortex in both intact and hypophysectomized animals, an effort has been made to discover whether adrenal hypertrophy can be dissociated from hypophyseal function under any conditions. *b*) We have attempted to learn whether or not there is a characteristic physiologic process that can be regarded as a common denominator of the many and apparently unrelated circumstances in which adrenal hypertrophy has been described. Obviously, a physiologic analysis of each of these circumstances has not been made, but some information has been obtained by correlating the more strictly morphologic material with the small amount of biochemical data that is available.

Administration of cortical steroids of the corticosterone type to experimental animals has been found to result in an elevation of carbohydrate levels of body fluids and tissues and an increased nitrogen excretion (Long, *et al.*, 1940). From these studies and others of the metabolism of adrenalectomized animals, the following have been suggested as the sites of action of the 'protein-carbohydrate' adrenal cortical steroids (Long, 1942, *b*): *a*) Increased catabolism of tissue proteins; *b*) Deamination of amino acids; *c*) Formation of glycogen from 3 or 6 carbon glycogen precursors; *d*) Inhibition of glycogenolysis; *e*) Inhibition of peripheral oxidation of glucose. The biochemical behavior of animals suspected of having an increased cortical hormone requirement was therefore examined in the light of these hypothetical sites of action of the hormone. The possibility that excessive salt loss or deprivation, or other disturbance in electrolyte metabolism, might constitute a stimulus to the adrenal cotex was also considered, but only a small amount of experimental evidence pertinent to this phase of the problem was found.

Compensatory Hypertrophy

A brief examination of the literature concerned with compensatory hypertrophy of adrenal tissue in a number of circumstances is a necessary foundation upon which a consideration of bilateral adrenal hypertrophy can be built. All workers agree that removal of one adrenal results in hypertrophy of the remaining one, or that following enucleation of both adrenals the cortical remnants quickly assume a mass which is roughly comparable to that present in the normal animal. To these well-known facts one must add information gained from a study of homotransplants, and the conditions under which they grow. All of the studies on compensatory hypertrophy (Winter and Emery,

1936; MacKay and MacKay, 1938; Swinyard and Bruner, 1940; Simmons and Whitehead, 1937; Golla and Reiss, 1941; Collip *et al.*, 1933; Shumacher and Firor, 1934; Baker and Bailiff, 1939; Ingle and Higgins, 1938, 1939) and those on homotransplants (Wyman and tum Suden, 1937; Turner, 1939; Kroc, 1942) in which there has been an investigation of this aspect of the problem are in remarkable agreement on one major point: it is universally accepted that hypertrophy does not occur and that homotransplants do not 'take' in the absence of the hypophysis. In addition, compensatory hypertrophy is suppressed by the administration of adrenal cortical extract, and, in the case of homotransplants, no 'takes' are obtained in animals which contain remnants of functioning cortical tissue. It has also been demonstrated that a measure which is known to cause bilateral hypertrophy of the cortex in intact animals accentuates compensatory hypertrophy in the unilaterally adrenalectomized animal.

Additional information about the mechanisms of adrenal hypertrophy is implicit in other experiments (Ingle and Fisher, 1938; Billman and Engel, 1939) in which vicarious action of fetal suprarenal glands is claimed. Animals newly born of adrenalectomized mothers show abnormally large adrenals, an observation which derives added significance from the fact that progesterone has been shown to cause adrenal atrophy (Clausen, 1940). Similarly, hypertrophy of the adrenals in the 'normal' partner following bilateral adrenalectomy in its parabiotic twin has been described (Li and P'an, 1940).

Adrenotropic Hormone

The problem of the pituitary-adrenocortical relationship recently has been the subject of a review by Swann (1940), to which the reader is referred for an extensive bibliography on this subject.

It is now well recognized that in almost all species hypophysectomy is followed by adrenal cortical atrophy (Houssay, Biasotti, *et al.*, 1933; Collip, *et al.*, 1933; Reese and Moon, 1938; Meyer, *et al.*, 1939; Anselmino, *et al.*, 1933; Atwell, 1935; Emery and Atwell, 1933; Miller and Riddle, 1939; Moon, 1940; Emery and Winter, 1934; Moon, 1937), while the administration of suitable extracts of the anterior pituitary gland will cause increased activity and hypertrophy of the adrenal cortex in both normal and hypophysectomized animals (Winter and Emery, 1936; Blumenfeld, 1939; Freudenberger and Howard, 1937; Freudenberger and Hashimoto, 1937; Lauson, *et al.*, 1937; Waterman, 1939; Hashimoto, 1940; Freudenberger and Billeter, 1935; Hatai, 1915; Billeter, 1937).

Earlier studies suggested that the adrenotropic effect of anterior pituitary extract was mediated through the thyroid and, indeed, the separate existence of a specific adrenotropic factor was doubted by some. Thus, a failure to observe adrenal hypertrophy after anterior pituitary extracts was noted in thyroidectomized rats (Emery and Winter, 1934) and guinea pigs (Rosen and Marine, 1939) or after the implantation of beef anterior pituitary into thyroidectomized rats (McQueen-Williams, 1934). Other workers (Atwell, 1937; Jores and Becker, 1937; Moon and Hansen, 1940), however, were able to

demonstrate adrenal hypertrophy in thyroidectomized animals. Since thyroxin will cause adrenal hypertrophy, the presence of thyrotropic factor in anterior pituitary extracts may cause some degree of adrenal hypertrophy if thyroid tissue is present. Moreover, it is known that many non-specific substances may produce adrenal hypertrophy in animals with intact hypophyses. Finally, the observations (Swann, 1940; Moon and Hansen, 1940; Ingle and Higgins, 1938) that hyperthyroidism in hypophysectomized animals does not produce adrenal hypertrophy, and the recent isolation and separation of the adrenotropic and thyrotropic factors in relatively pure form, make it appear most unlikely that the pituitary control over the adrenal is mediated through the thyroid.

Since it is well established that there exists a specific adrenotropic principle in the anterior pituitary gland, it becomes of great importance to determine, if possible, to what degree adrenal hypertrophy caused by diverse factors is mediated through the pituitary. Experiments with homotransplants of adrenal cortical tissue and compensatory adrenal hypertrophy after unilateral adrenalectomy demonstrate that the anterior pituitary is necessary for these processes. Moreover, those stimuli which increase compensatory hypertrophy are ineffective after hypophysectomy. Such stimuli as estrogens, toxins, tumors, thyroxin and goitrogenic diets do not produce adrenal hypertrophy in hypophysectomized animals, even if, as was done in some cases, sufficient adrenotropic hormone is given to prevent the usual atrophy following hypophysectomy. An exception, however, is found in the pigeon, in which Miller and Riddle (1939, 1941) have produced adrenal hypertrophy with the thyroid substance and insulin even after hypophysectomy, while estrone was ineffective after hypophysectomy. Whether this is evidence for a certain degree of adrenal autonomy, or whether it is simply a species peculiarity is difficult to say. That the adrenal can exercise a measure of independence is shown by the fact that the hypophysectomized animal does not exhibit evidence of total adrenal insufficiency, as would be anticipated were the adrenal completely dependent on the pituitary. It is of considerable interest, particularly in view of the concepts concerning the metabolic stimuli for adrenal hypertrophy to be developed in this review, that the greatest degree of derangement in cortical function after hypophysectomy is in protein and carbohydrate metabolism. Mineral metabolism is less impaired in hypophysectomized than in adrenalectomized animals.

Recent experimental work suggesting that variations in adrenal cortical size may occur in the absence of the pituitary have been reported by Bourne and Zuckerman (1940) and Sarason (1943). The former authors demonstrated that the rhythmic variations in adrenal size known to occur during the estrous cycle occurred in hypophysectomized spayed rats during artificial estrous cycles induced by threshold amounts of estrogen. Large doses of estrone, however, do not produce adrenal hypertrophy in hypophysectomized rats. Sarason has shown that the administration of desoxycorticosterone to hypophysectomized rats will result in even greater atrophy of the cortex than ensues from hypophysectomy alone. This is of interest in view of the concept

held by Ingle, *et al.* (1938), and Wells and Kendall (1940) that the adrenal cortical atrophy following the administration of large doses of cortical hormone to normal animals is brought about by suppression of pituitary adrenotropic hormone secretion. Finally, Albert and Selye (1942) have recently reported that desoxycorticosterone prevents the adrenal hypertrophy following estradiol, but does not affect pituitary size. Taken as a whole, the experimental evidence would suggest that while the adrenal may maintain a certain degree of freedom from pituitary control (which may be complete in the case of the estrous cycle variations), adrenal hypertrophy is mediated through the pituitary adrenotropic hormone in most cases.

The problem of the extent of hypothalamic control over hypophyseal function has been reviewed by Uotila (1940). This investigator found no change in weight or histologic appearance of the adrenals in animals with the pituitary stalk sectioned. In addition, the adrenal response of such animals to cold exposure was comparable to that of normal animals when the relative thyroid insufficiency of animals with the transected pituitary stalks was compensated for by thyroxin administration. Adrenals of stalk-sectioned animals also respond normally to the administration of estrogen. From these studies Uotila concludes that the adrenocorticotropic hormone is under humoral control. Swinyard (1937) failed to find neural connections with the cells of the adrenal cortex of the cat or man.

Recent attempts at purification of the adrenotropic hormone have been made by Astwood (1942), Li, *et al.*, (1942) and by Sayers, *et al.*, 1943. The former has succeeded in obtaining very small yields of a substance with marked adrenotropic activity and without appreciable growth, thyrotropic or gonadotropic activity. Both investigators are agreed that the adrenotropic hormone content of pig pituitary gland is much higher than that of any other species examined.

ADRENAL, ANTERIOR PITUITARY AND GONAD INTERRELATIONSHIPS

Sex differences in adrenal size. The relationship between the adrenal cortex and the gonads has been the subject of a large and perplexing literature. However, recently several important contributions have been made which help to clarify this confusing subject. Not least in importance has been the greater understanding of the metabolic changes produced by the adrenal cortical and sex steroids.

In all species studied, except the pigeon (Riddle, 1923) in which the male adrenal is slightly larger than the female, a consistent weight difference has been found between the adrenals of males and females, the gland being heavier in the latter. Jackson (1913) first noted that for rats with body weights above 100 gm. there was a linear relationship between body weight and adrenal size and that the glands of females were consistently heavier than those of males. This sex difference was confirmed by Donaldson (1924) and by Cole and Harned (1942) in two strains of rats, by Deanesly (1938) in mice and Bennett (1940), in cats. Cole and Harned report a similar sex difference in the pituitary weight-body weight ratio, which is of great interest in view of the

report that pituitaries of female rats, guinea pigs and cattle are richer in adrenotropic hormone (Emery and Winter, 1934) and that more adrenal cortical hormone is necessary to produce adrenal atrophy in female rats than in males (Ingle, 1939). These studies constitute further evidence for the intimate relation between pituitary and adrenal cortical activity. Since this discrepancy in weight is not seen in rats weighing less than 100 gm. it seems likely that estrogen secretion by the ovaries may be one factor in the greater adrenal and pituitary size of female rats.

Estrous cycle, ovulation and mating season. Cyclic variations in adrenal size have been observed in female rats and mice by several workers (Masui and Tamura, 1926; Billman and Engel, 1939; Bourne and Zuckerman, 1940; MacKenzie and Nahm, 1934). This variation is equal to about 3 to 4 mg. per 100 gm. of body weight in rats. The maximum weight is achieved at estrus, although Andersen and Sperry (1937) noted the greatest weight during the proestrous phase. In rats without estrous cycles no such variation occurs (Bourne and Zuckerman, 1940; Andersen and Kennedy, 1932). Although most earlier workers thought that these changes were mediated through estrogenic stimulation by the gonads, Bourne and Zuckerman (1940) and Zuckerman, *et al.* (1938) have recently presented evidence suggesting that this is an autonomous variation in size, independent of both gonads and pituitary and that the adrenal cortex exerts a direct effect on the accessory reproductive organs. These workers found that spayed rats, in which artificial estrous cycles were induced by the administration of small daily doses of estrogen, showed a fluctuation in adrenal size similar to that in intact animals during the normal estrous cycle. Moreover this cyclic variation was found also in the hypophysectomized rat during artificial cycles induced with minimal amounts of estrogen. This observation takes on added interest in light of an earlier report by Shumacker and Firor (1934) that the pituitaries of adrenalectomized rats showed a decrease in gonad stimulating power. Moreover, Wooley, *et al.* (1939) and Gardner (1941) in the course of studies on the effect of gonadectomy on tumor formation in mice, report that if the animals are overiectomized early in life, some develop striking adrenal hypertrophy, and in some cases true adrenal cortical tumors, which are associated with definite evidences of estrogenic stimulation of the animals as determined by the condition of the pelves, uteri and mammary glands. The observations of Bourne and Zuckerman (1940) describe one type of fluctuation in adrenal size which may not be dependent on the pituitary gland.

In other species cyclic variations in adrenal size have also been reported. Riddle (1923) described a 40 per cent increase in adrenal size at the time of ovulation in pigeons. A similar change has also been reported in the frog (Stillung, 1898). In the squirrel (Zalesky, 1934) and the mole (Kolmer, 1918) increases in adrenal size have been noted during the mating season in both sexes, but the changes are not impressive. Under all these circumstances increased activity may be a factor in increasing the adrenal size.

Castration. In the male animal all reports (Winter and Emery, 1936; Hall and Korenchevsky, 1938; Korenchevsky *et al.*, 1937; Lawless, 1936; Zeckwer,

1938) are in agreement that castration is followed by adrenal and pituitary hypertrophy. The adrenal hypertrophy was found to be due to an increase in the size of the zona fasciculata and reticularis. Male sex steroids (Hall and Korenchevsky, 1938; Korenchevsky, *et al.*, 1936) inhibit this increase and cause vacuolization of the cells and a decrease in the lipoid of the reticularis and fasciculata.

The literature has been confusing concerning the effect of castration on adrenal size in female animals. Most authors (Winter and Emery, 1936, Blumenfeld, 1939; Freudenberger and Howard, 1937; Freudenberger and Hashimoto, 1937; Lauson, *et al.*, 1937; Waterman, 1939; Hashimoto, 1940; Freudenberger and Billeter, 1935; Hatai, 1915; Billeter, 1937) have reported a decrease in adrenal size after spaying, but others reported either no change (Lauson, *et al.*, 1937) or an increase (Bourne and Zuckerman, 1940; Hashimoto, 1940; Zukerman, *et al.*, 1938; Wooley, *et al.*, 1939; Andersen and Kennedy, 1933; Chiode, 1938). Hashimoto (1940) has re-examined this problem recently and finds that the response depends on the age of the animal at the time of ovariectomy and on the time after operation that the animal is examined. Ovariectomy in immature rats at 26 days of age produced initial hypertrophy at 5 to 6 weeks, followed by a progressive decrease in the adrenal weight/body weight ratio. From the thirteenth to the thirty-ninth week an actual decrease in the absolute adrenal weight took place. After the sixth week the spayed rats grew more rapidly than the controls. Andersen and Kennedy (1933) found hypertrophy one week after ovariectomy in mature rats and atrophy after 3 weeks, while no changes were noted in immature rats examined at 7 or 85 days. Chiode (1938) noted hypertrophy of adrenals in immature rats between 45 and 70 days, followed by progressive atrophy thereafter up to 100 days. The discordant results in the literature are thus explained by the fact that different investigators examined their animals at different times after ovariectomy.

Estrogens and androgens. Studies on the effects of both natural and synthetic estrogens on pituitary and adrenal size have, in almost all cases, revealed a markedly stimulating influence. This effect has been reported in mice (Burrows, 1936), rats (Golla and Reiss, 1941; Janes and Nelson, 1942; del Castillo and Sammartino, 1937; Ellison and Burch, 1936; Loesser and Erbacher, 1939; Ingle, 1941; Tuchmann, 1936; Selye and Albert, 1942; Albert, 1942; Morrell and Hart, 1941; guinea pigs (Tuchmann, 1936), pigeons (Hashimoto, 1940), and chickens (Brenemen, 1942). Exceptions to these findings are the reports of Clausen and Freudenberg (1939) and Selye and Albert (1942) who found that estrogens cause a decrease in adrenal and pituitary size in immature intact and castrated rats. As with castration in the immature female rat, there is apparently a period in the development of the animal in which the effect is the reverse from that in the mature animal. Lauson, *et al.* (1937) described a 28 to 46 per cent increase in pituitary size but no change in the adrenals after estrin administration to mature ovariectomized rats. Striking hypertrophy of the adrenals may be produced by the administration of the estrogens and the effect is more marked with moderate-

sized doses than larger ones (Bourne and Zuckerman, 1940). Excessive dosage may account for the reported failures in some cases. In all species studied, hypophysectomy prevents the hypertrophy after estrogens, even when sufficient adrenotropic hormone is given to prevent the usual post-hypophysectomy adrenal atrophy (Golla and Reiss, 1941; Ellison and Burch, 1936; Selye and Collip, 1936). If testosterone is administered simultaneously with the estrogen, both the adrenal and the pituitary hypertrophy are inhibited (Albert, 1942). When estrogens are administered together with other factors known to produce hypertrophy, such as thyroid substance (Korenchevsky and Hall, 1941), or after unilateral adrenalectomy (Golla and Reiss, 1941), a much greater effect is produced than with either alone. Thus Golla and Reiss report a 250 per cent increase in compensatory hypertrophy after estrone as compared to 60 per cent without estrone.

In contrast to the estrogens, progesterone has been found to produce adrenal atrophy in male rats (Clausen, 1940). This is not surprising in view of the structural similarity between progesterone and certain of the adrenal cortical steroids and the fact that adrenalectomized animals may be maintained with progesterone.

The reports on the effect of androgens on adrenal size are less consistent than those on the estrogens. However, except for that of Nathanson and Brues (1941), who found increased mitotic activity in the adrenal of immature female rats, all are in agreement that androgens either have no effect or produce atrophy of both adrenals and pituitary glands. Thus Mazer and Mazer describe atrophy in mature and immature female rats (1939) and Selye (1939) describes similar changes in mice while Bottomley and Folley (1938) in guinea pigs and Clausen and Freudenberger (1939) in immature female rats, noted no influence of crystalline androgens. Schilling and Laqueur (1942) have shown that the decrease in adrenal size in female rats is greater at diestrus than at estrus, and that body weight increase is greater at the former period. Of interest are the reports (Hall and Korenchevsky, 1938; Korenchevsky, *et al.*, 1937; Korenchevsky and Dennison, 1936) that the androgens will inhibit castration hypertrophy of the adrenals in male rats and will inhibit the hypertrophy after estrogens (Albert, 1942) and after thyroid (Korenchevsky and Hall, 1941).

Recent studies on the rôle of the adrenal cortex, anterior pituitary and gonads in protein and carbohydrate metabolism aid in our understanding of the morphologic variations in these endocrine glands. The adrenal cortex has been shown to be intimately related to protein metabolism (Long, *et al.*, 1940; Long, 1942). Administration of potent adrenal cortical extract or of cortical steroids with an oxygen atom on carbon 11 to normal or adrenalectomized animals causes an increase in protein catabolism and gluconeogenesis as manifested by a rise in urinary nitrogen excretion and increased liver glycogen. By increasing protein catabolism, growth is suppressed (Wells and Kendall, 1940). Adrenalectomy is followed by changes in the reverse direction. Similar changes can be brought about by stimulation of the adrenal by adrenotropic hormone (Grattan, *et al.*, 1941). In direct contrast to these effects

on protein metabolism are those produced by the growth hormone of the anterior pituitary (Long, 1942). This latter hormone increases protein anabolism and with this there is a decrease in urinary nitrogen excretion and an increase in body growth. That the growth hormone and the adrenal cortical hormone may have a reciprocal relationship to each other with respect to their effects on protein metabolism is suggested by the recent work of Winternitz (1942). This observer found that the gain in weight of adrenalectomized rats maintained on saline was similar in character to that of rapidly growing animals; *i.e.*, a higher content of water, protein and ash and a lower content of fat ('chemical' growth), while the pair-fed controls showed smaller proportions of water, larger amounts of fat and little change in protein and ash content. These results in adrenalectomized animals were very similar to those reported by Lee (1936) for animals treated with growth hormone. These findings, together with the known fact that immediately after hypophysectomy and before adrenal atrophy occurs, there is an increased nitrogen excretion (Long, 1942) suggest to Winternitz that the removal of those tissues which normally promote protein catabolism (adrenal cortex) results in a relative hyperactivity of the gland involved in protein anabolism, the anterior pituitary, while the reverse is true after hypophysectomy.

That the metabolic effects of the estrogens and the androgens may be mediated through the anterior pituitary (by the adrenotropic hormone and the growth hormone, respectively) is suggested by the following facts.

The estrogens, particularly diethylstilbestrol, have recently been shown to exert an effect in normal rats which is indistinguishable from that of the adrenal cortical hormone (Long, 1942; Anselmino, *et al.*, 1933; Janes and Nelson, 1942; Ingle, 1941; Griffiths, *et al.*, 1941; Fry, *et al.*, 1942). Thus, they cause increased nitrogen excretion and an increase in the liver nitrogen, glycogen and weight. However, this effect does not appear in the adrenalectomized animals (Long, 1942; Fry, *et al.*, 1942) or in the hypophysectomized animals, demonstrating that it is a manifestation of increased adrenal activity, which in turn is mediated through the adrenotropic hormone of the anterior pituitary. Further, the administration of estrogens is associated with weight loss and a suppression of growth (Albert, 1942; Morrell and Hart, 1941, *b*; Richards and Keuter, 1941; Spencer, *et al.*, 1931; Matthews, *et al.*, 1942). Richards and Keuter (1941) have recently demonstrated that the administration of growth hormone will prevent this inhibition of growth by estrogens.

Studies on the metabolic relationships of the androgens have given less clear-cut results but they are nevertheless quite suggestive of an opposite effect. Several workers (Kochakian and Murlin, 1935; Kenyon, *et al.*, 1938; Rubenstein and Solomon, 1940; Kenyon, *et al.*, 1940; Shay, *et al.*, 1941) have reported increase in body weight in rats, dogs and man after the administration of androgens. Although part of this could be attributed to water retention, evidence for increased protein anabolism was found in castrated dogs (Kochakian and Murlin, 1935) and in normal and eunuchoid men (Kenyon *et al.*, 1938, 1940) after androgen administration. A pronounced nitroten retention was noted and in addition a decrease in blood urea and non-protein nitro-

gen similar to that seen after the administration of growth hormone, In contrast to this is the report of Sandberg, *et al.* (1939) who observed a reduction in the retention of nitrogen, sulfur and potassium after castration in the rapidly growing 7-week-old male rat. On the basis of such reports, Shay, *et al.* (1941) suggest that the androgens stimulate the production of growth hormone by the pituitary, while estrogens inhibit it.

Correlating these observations with the previously described morphologic changes, certain relatively clear delineations may be made. Thus we see that the administration of estrogens causes pituitary and adrenal hypertrophy and with this is evidence of increased adrenal cortical activity (increased protein catabolism and suppression of growth). The androgens produce a reduction in adrenal and pituitary size with evidences of increased protein anabolism and acceleration of growth. Here, with the adrenal atrophy one may speculate that an increase in growth hormone activity may become possible. Castration in the male rat produces adrenal and pituitary enlargement and with it suggestive evidence of increased protein catabolism (Sandberg, *et al.*, 1939); *i.e.*, predominance of the adrenal gland in protein metabolism. Castration in the female produces the reverse effect on the adrenal, namely atrophy. Unfortunately here we have no adequate metabolic data although weight gain has been noted by some authors after castration (Hashimoto, 1940 Sandberg, *et al.*, 1939).

In both their morphologic and metabolic effects the androgens and estrogens (and deficiency of each) are seen to be antagonistic and this is expressed in terms of protein metabolism, as an antagonism between the adrenal cortical hormone and the growth hormone. It should be emphasized at this point that, in contrast to factors described elsewhere in this review in which increased protein catabolism appears to be the stimulus for adrenal hypertrophy, here we are probably dealing with a direct stimulation of adrenal cortical activity which results in increased protein catabolism and increased gluconeogenesis.

From these studies certain clinical correlations might also be made. Hyperplasia or tumors of the adrenal cortex are associated with two syndromes. In one, Cushing's syndrome, there is evidence of an increase in true cortical activity; *i.e.*, mild diabetes which is often insulin resistant, and in a few cases that have been studied from this standpoint, increased nitrogen excretion. Albright, *et al.*, in a recent paper (1941) have emphasized hyperglyconeogenesis as a manifestation of this syndrome and points to the thin skin, easy bruisability, weak muscles and osteroporosis as evidences of protein deprivation. In the second syndrome, the adreno-genital syndrome occurring in children, we find that the adrenal is secreting large amounts of androgenic steroid, and occasionally very little of the so-called metabolism-regulating hormones of the adrenal cortex as is apparent from the fact that these patients sometimes die in typical Addisonian crisis. These cases are characterized by the absence of diabetes and the presence of sexual and somatic precocity (true growth). Albright treated several cases of Cushing's syndrome with testosterone propionate and demonstrated a prompt retention in nitrogen and phosphorus and with this, very suggestive clinical improvement. With large doses of estradiol-

benzoate in one case, on the other hand, a negative nitrogen balance was produced. The metabolic characteristics of these two syndromes and the results obtained in Cushing's disease after androgens and estrogens are quite consistent with the concept of the adrenal-gonad-pituitary relationship developed above.

The inter-relationships of the adrenals, the pituitary and the gonads are summarized in table I.

TABLE 1. PITUITARY-GONAD-ADRENAL RELATIONSHIPS IN PROTEIN METABOLISM

	ADRENAL CORTICAL EXTRACT	ADRENAL-ECTOMIZED	'GROWTH' HORMONE	HYPOPHYSECTOMIZED	ESTROGEN	OVARIECTOMY	ANDROGEN	CASTRATION
Adrenal size	−			−	+	−	−	+
Pituitary size	?	?	−?		+	−	−	+
N excretion	+	−	−	+	+	?	−	+
Growth[1]	−	+	+	−	−	+?	+	−

[1] The chemical composition of gain in weight has been used as the criterion of growth. True growth is characterized by synthesis of protein. Overall gain in weight may occur without true growth; *i.e.*, as in fat or salt and water retention.

Experimental Hyperthyroidism

Thyroxin and desiccated thyroid substance have been reported to produce adrenal enlargement in rats (Ingle and Higgins, 1938; Korenchevsky and Hall, 1941; Cameron and Carmichael, 1920; Cohen, 1935; de Wesselow, *et al.*, 1938; Nelson, 1942; Schmidt and Schmidt, 1938; Ingle and Kendall, 1938; Lowenstein and Zwemer, 1942), dwarf mice (Kemp and Marx, 1937), guinea pigs (Hoen and Oehme, 1938; Hoskins, 1910), rabbits (Squier and Garfield, 1922) and pigeons (Miller and Riddle, 1939). Again, with the exception of the pigeon, the effect is dependent on the integrity of the anterior pituitary. Thyroxin will greatly enhance the hypertrophy after unilateral adrenalectomy (Ingle and Higgins, 1938), after exercise (Ingle and Kendall, 1938) or after estrogen, while in turn hypertrophy produced by thyroxin is partially inhibited by androgens (Korenchevsky and Hall, 1941). Hoen and Oehme (1938) report that lactoflavin will inhibit thyroxin-induced adrenal hypertrophy and attribute the hypertrophy to lactoflavin depletion brought about by the hypermetabolism. However, these experiments were poorly controlled and lack confirmation. Iodides are without effect on adrenal size (Cameron and Carmichael, 1920).

The recent report of Lowenstein and Zwemer (1942) throws new light on the significance of adrenal hypertrophy after such a stimulus as thyroxin. These authors found that rats with hypertrophied adrenals actually may show a decreased resistance to intraperitoneal injections of potassium chloride while they were receiving the desiccated thyroid, but after the thyroid was stopped and before the adrenal hypertrophy had begun to regress there was an increased resistance to this toxic substance. From this observation one may speculate that while there is adrenal hypertrophy and, presumably, increased hormone production in response to the increased load due to excess protein breakdown because of the thyroxin, the body (through the adrenal

cortex) cannot endure the added insult of the toxic agent. However, when the maximum thyroxin effect on the tissue has been withdrawn, the hypertrophied and now hyperfunctioning glands are able to meet the metabolic situation created by the toxic substance more effectively than are normal glands.

De Wesselow and Griffiths (1938) pointed out the fact that the adrenal glands do not undergo hypertrophy in animals given dinitrophenol, although the total metabolism of such animals may be as high as that of thyroxin-treated ones. This finding is of extraordinary interest in view of the reports of Dunlop (1934) and Tainter, Cutting and Hines (1935). These workers have shown that the accelerated energy production caused by dinitrophenol is not attended by an increase in nitrogen metabolism comparable to that which accompanies a similar increase in energy expenditure provoked by the use of thyroid hormone.

In the adrenal hypertrophy produced by thyroxin we find evidence for the possibility of increased protein catabolism being the stimulus for adrenal hypertrophy. The experimental work of Wells and Kendall (1940) lends added weight to such a concept. These workers found that thyroidectomy diminished the amount of glucose and nitrogen excreted by phlorizinized rats, but that the rate of gluconeogenesis could be restored to normal by thyroxin. Adrenalectomized, phlorizinized rats showed an even greater degree of depression of gluconeogenesis and this could be restored to normal by compound 'E' (adrenal cortical steroid). Adrenalectomy and thyroidectomy together resulted in the lowest rate of glucose formation and nitrogen excretion and this could not be restored to normal by compound E alone, but could be made normal or greater if thyroxin were added. In other words thyroid deficiency depresses protein catabolism to such a degree that compound E cannot exert its full effect on protein metabolism while thyroxin administration increases protein breakdown so that a greater gluconeogenetic response is possible after compound E.

Kennedy and Purves (1941) describe pituitary and adrenal hypertrophy in rats fed goitrogenic diets. This response does not occur in hypophysectomized rats.

Cold

Some of the evidence involving the rôle of the adrenal cortex in the response to cold is of a negative sort; *i.e.*, it is universally agreed (for example, see Wyman and tum Suden, 1929; Weiser and Norris, 1936; Horvath, 1938; Tyoslwitz and Astwood, 1942) that adrenalectomized (or hypophysectomized) animals are unable to resist exposure to a cold environment as well as intact animals do. However, adrenal hypertrophy following exposure to cold has also been reported (Selye, 1936, 1937; Emery, *et al.*, 1940; Bernstein, 1941) and the histochemical changes associated with exposure to extremes of temperature has been studied (Flexner and Grollman, 1939). Although little data on food consumption during cold exposure has been presented, other workers find that there is an increase in voluntary food intake in rats exposed to cold, but in spite of this these animals gain weight at an abnormally slow rate (Schwabe, *et al.*, 1938; Mills, 1941). A possible clue to one of the factors un-

derlying the adrenal hypertrophy is the fact that the thyroid gland undergoes marked hypertrophy during exposure to cold. Since there is a markedly increased oxygen uptake, this hypertrophy is indicative of hyperactivity of the thyroid. It thus becomes possible to associate enlargement of the adrenals due to exposure to cold with that found following the injection of thyroid preparations. In addition, it is not al all unlikely that, as a result of the cold environment, the oxidative mechanisms which yield the energy for the continuous synthesis of peripheral tissue protein may be sufficiently disordered so that a transitory preponderance of protein breakdown over protein synthesis may occur. This hypothesis could be tested by a study of the effect of cold exposure upon the nitrogen equilibrium of experimental animals.

One of the studies, on the resistance of animals to cold, that of Tyslowitz and Astwood (1942), is noteworthy because it gives some information on the rapidity with which the adrenal may respond. These investigators found that either crude saline anterior pituitary extract or purified corticotropin increased the resistance of hypophysectomized rats to cold, and that this effect was readily apparent after one injection of an effective extract.

Heat

There is no convincing evidence that heat alone produces adrenal hypertrophy, although the diminished resistance of adrenalectomized animals to heat has been established (Hartman and Lockwood, 1932). Rats bred for 8 generations under tropical conditions of temperature and humidity showed a relative increase in adrenal size. Other studies (Schmidt and Schmidt, 1938; Flexner and Grollman, 1939; Bernstein, 1940, 1941; Clark, 1938; Cramer, 1928) have emphasized the histology and histochemistry of adrenals of animals exposed to heat, but few gland weights are recorded. Since exposure to heat results in a dimunition in spontaneous food intake in the rat this factor would have to be well-controlled in any study of the effect of heat on the adrenals.

Exercise

The mechanisms involved in the production of adrenal hypertrophy during muscular exercise, first studied by Donaldson and Meeser (1933, 1938) are only indifferently understood. The basis facts in the case are these: the adrenalectomized animal does not tolerate exercise well; the tolerance of the adrenalectomized animal can be restored by the administration of suitable cortical extracts; and intact animals forced to exercise show a considerable hypertrophy of the adrenals within as short a time as 12 hours after beginning work. Ingle (1938) has shown that no hypertrophy appears in exercised animals if they are either hypophysectomized or treated with adrenal cortical extract. Beznak and Korenyi (1941) have claimed that hypertrophy of the cortex caused by exercise is reduced by desoxycorticosterone. However, Ingle (1940) has shown that this steroid does not correct the 'work deficiency' of adrenalectomized animals. Hasch and Hajdu (1939) suggest that the hypertrophy of the cortex observed after exercise is caused by the hyper-

cholesterolemia attendant on exercise, but other workers have stressed the fact that the hypertrophy of exercise is characteristically associated with lipoid (Dosne and Dalton, 1941; de Jongh and Rosenthal, 1933) and, especially, cholesterol depletion (Knouff, *et al.*, 1941) of the gland. The situation is further confused by the claims of Beznak and Perjes (1935a) and Perjes (1937) who believe that the hypertrophy during work is due to a relative deficiency of vitamin B complex and state that the enlargement can be prevented by the administration of 5 times the normal vitamin B requirement. An analysis of mechanism in this complicated aspect of the problem must begin by pointing to the enormous acceleration of many of the energy-producing reactions of the body and to the rapid breakdown to its protein tissues. Thus, superficially, at least, there is a resemblance between the effects of cold, exercise and thyroxin administration, although it has been shown that the combination of work and thyroxin exerts an additive effect, and results in a greater hypertrophy than does either alone (Ingle and Kendall, 1938).

Pregnancy, Puerperium and Lactation

During the early part of pregnancy there may be some slight increase in adrenal size, but the reports are not in agreement on this point (Bennett, 1940; Andersen and Sperry, 1937; Andersen and Kennedy, 1932; Caussade, 1938; Hewitt and van Liere, 1941; Randall and Graubard, 1940). All studies, however, agree that in late pregnancy, and especially in the puerperium, a striking increase in adrenal size may occur in rats (Andersen and Sperry, 1937), guinea pigs (Hewitt and van Liere, 1941; Sleeth and van Liere, 1939) and rabbits (Randall and Graubard, 1940). An increase of as much as 34 per cent has been noted as early as 72 hours post partum by Sleeth and van Liere (1939) who attributed it to fluid distribution in the gland itself since they felt 72 hours to be too short a time for hypertrophy to develop. However, ample evidence has been presented elsewhere in this review to show that hypertrophy may occur with great rapidity, sometimes within 12 hours.

The rapid development of adrenal hypertrophy immediately post partum suggests that the absorption of protein breakdown products may be the stimulus. The largest adrenals seen in this laboratory have been found in pregnant diabetic rats in which fetal absorption was taking place.

The report of Brody, *et al.*, (1938) on the energy-metabolism levels of experimental animals during gestation and lactation reveals that with the onset of lactation there is a sudden and striking increase both in spontaneous food intake and total metabolism, the latter often reaching a level twice that observed during gestation. It is possible that this hypermetabolism may contribute to the increase in adrenal size observed during the puerperium.

Urine of pregnant women has also been found to stimulate adrenal hypertrophy (de Boissezon and Peyrot, 1934; de Boissezon, 1936). We are unable to interpret these results. Golla and Reiss (1942) have recently reported that pregnant mare's serum will increase the adrenal weights of hypophysectomized rats and 9-day-old chicks. They postulate the presence in the pregnant mare's serum of a new corticotropic factor.

Inanition

The literature on the effect of diet and dietary insufficiency is confusing, but seems to be in agreement on one point; namely, that complete inanition will produce adrenal hypertrophy (Selye, 1936; Sarason, 1943, *b*; Beznak and Korenyi, 1941; Jackson, 1915; Chila, 1938; Mulinos and Pomeranz, 1940, 1941; Mulinos, *et al.*, 1942). Two workers (Andersen, 1935; Olsen and Bloor, 1941) report no changes in adrenal weight after a 24-hour fast, although others (Sarason, 1934, *b*), have demonstrated histologic changes this early. Starvation hypertrophy is not prevented by vitamin B_1, a deficiency of which is also known to produce adrenal hypertrophy. The metabolic changes associated with fasting need no detailed discussion. Complete inanition makes it necessary for the organism to call on its protein stores in order to maintain carbohydrate levels. The liver glocogen of fasted rats reaches its lowest point at about 24 hours and thereafter increases somewhat, presumably due to gluconeogenesis from protein (Mirsky, *et al.*, 1938).

In contrast to these clear-cut effects of starvation are the interesting reports of Mulinos and Pomeranz (1940, 1941) and Mulinos, Pomeranz and Lojkin (1942) on the effects of underfeeding on organ size. These workers find that if rats are chronically underfed there is a decrease in adrenal weight which is relatively greater than the loss in body weight. If these animals are refed, the adrenals return to normal size and if they are subjected to complete inanition the adrenals increase in size. Implantation of pituitary glands from normal rats into the chronically underfed rats results in a weight gain by the atrophied adrenals. These results would suggest that the underfeeding has produced a general lowering of the metabolic level of the organism and with it decreased function of many endocrine glands. Other workers have shown a distinct decrease in metabolic rate in chronically underfed animals or animals on low protein diets. A deficiency of adrenotropic hormone does not appear to be likely, since the animals seem to be able to respond promptly to starvation by an increase in adrenal size.

Insulin Administration

In rabbits (Schenk and Langecker, 1935; Randall, 1940), mice (Mikeleitis, 1940) and pigeons (Miller and Riddle, 1941; Riddle, *et al.*, 1924) adrenal hypertrophy has been reported after prolonged administration of insulin. Thatcher (1926) failed to produce changes in rabbit adrenals, but he used quite small doses. In pigeons, which are quite resistant to the hypoglycemic effect of insulin, measurable adrenal enlargement was noted as early as $8\frac{1}{2}$ to 40 hours after large doses of insulin. Moreover, hypophysectomy in this species did not prevent the response.

With the increased carbohydrate utilization brought about by insulin and the depletion of liver glycogen, an attempt is made by the organism to compensate for this transitory carbohydrate lack by increasing glyconeogenesis from protein; *i.e.*, by increased protein catabolism. This appears to be the most likely stimulus for adrenal hypertrophy after insulin, although it might be maintained with equal justice that the depletion of carbohydrate stores itself is the stimulus.

The condition of insulin hypoglycemia is an excellent example of a circumstance in which it not feasible to judge the level of activity of the adrenal cortex by determinations of the tissue and fluid carbohydrate levels. For here the peripheral utilization rate of glucose is so rapid that the tissue carbohydrate stores may be depleted as rapidly as they are formed, and the rate of nitrogen excretion becomes the only true estimate of the rate of protein breakdown.

Pancreatic Diabetes

Little reliable data on adrenal size in diabetes is available in published reports. Clinically (Sarason, unpublished) the picture is too often complicated by infections and other diseases to make interpretation of the results worthwhile. An attempted analysis of the data on the adrenal weights of diabetic rats was disappointing, inasmuch as the diabetes varied in severity over a wide range, and, in addition, the animals had often been treated with insulin or anterior pituitary extracts. It is of some interest, however, that the largest rat adrenals seen in this laboratory were those of three pregnant diabetic rats which were resorbing litters at the time of death. The paired glands weighed 160, 175 and 189 mg., respectively, and the adrenal/body weight ratios in gm./kg. were approximately 0.6.

Phlorizin Diabetes

Unpublished data of Evans and Long (personal communication) contain records of marked adrenal hypertrophy in chronically phlorizinized rats. The excessively rapid protein breakdown in these animals requires no comment.

Anoxia

In 1934 and 1936 Evans reported that rats exposed to one-half atmospheric pressure exhibited a marked increase in blood sugar and liver glycogen levels, that these phenomena were associated with increased nitrogen excretion, and that this whole sequence of events did not occur after removal of the adrenal glands. Inasmuch as there was no obvious difference in the behavior of adrenodemedullated animals and that of intact ones, Evans concluded that the adrenal cortex was necessary for adaptation to anoxia. Hypertrophy of the adrenals in animals exposed to low pressure has been reported by Armstrong and Heim (1938) and by Langley and Clarke (1942), and in animals poisoned with repeated small doses of cyanide by Messerle (1927).

Recently there has been a revival of interest in this field, and, in fact, during the past 6 months two groups of investigators have reviewed the literature and presented their own theories on the rôle of the adrenal cortex in acute and chronic anoxia (Langley and Clarke, 1942; Lewis, *et al.*, 1942). This aspect of the study of adrenal hypertrophy more than any other has produced a concerted effort to attack the problem in terms of fundamental physiologic and biochemical mechanisms.

After an exhaustive study of the response of several species to anoxia, Lewis, Thorn, Koepf and Dorrance (1942) conclude that during the early phase of anoxia there is an acceleration in the rate of utilization of carbohydrate and a rapid depletion of the carbohydrate stores of the body. This, in

turn, produces activation of the adrenal, and very soon afterward carbohydrate from protein is supplied to the organism is abnormally large amounts. They imply that the adrenal is stimulated by the increase in rate of glucose utilization, but they do not suggest any possible mechanism through which this stimulus might act.

Langley and Clarke (1942) criticize the views of Lewis and his colleagues and present an alternative theory of their own. They state: 'The activity of the kidneys in compensating for the alkaline shift caused by the hyperventilation in response to anoxemia suggests that the adrenal-renal relationship is important in the adaptation of the organism to reduced pressures.' Nims (personal communication) is also struck by the changes in acid-base equilibrium that are incident to high altitude exposure, and he believes that the activating mechanism of the adrenals may be related to alterations in the pH of the body fluids. It is of some interest to note that both Lewis and his colleagues and Langley and Clarke recognize the fact that one of the characteristic features of the response to anoxia is increased formation of glucose from protein.

The evidence for an increased hormone requirement and secretion during adaptation to anoxia is good, though indirect. Langley found that adrenalectomized animals exposed to half an atmosphere of pressure required many times the dose of cortical extract required for maintenance at sea-level. As the animals became adapted to the low pressure it was found that they could be maintained with progessively diminishing doses of extract. Similarly, Sarason (1943, *a*) reports that during the first 5 days of exposure of intact rats to anoxia of the same degree he finds lipoid-depletion of the adrenals which he believes to be indicative of activation, but by the seventh or eighth day of exposure the lipoid pattern of the glands has reverted to normal. These observations suggest that during the first few days of adaptation there is an increased hormone output, but that hormone production tends to return to normal as adaptation progresses.

Dohan (1942) studied the effect of anoxia on small groups of animals fed diets high in carbohydrate, fat and protein, respectively, for several days before exposure. He found no difference in their response to anoxia, nor did he notice variations in adrenal size in animals on different diets. (For a report of the possible rôle of anoxia in the maintenance of fetal adrenal size, see Goldzieher, 1934.)

Shock and Burns

The relation of the adrenal cortex to shock has recently been reviewed by Rubin, Kennedy and Ralli (1941) and the histology of this relationship has been studied by Donahue and Parkins (1935). The adrenal cortex enlarges in response to a great variety of shock-producing agents, but the rôle of the cortex in the resistance of the organism to shock is poorly understood. From the point of view of the metabolic disorders that are associated with shock, the time relationship of the adrenal enlargement to the administration of the shock-inducing procedure seems to us to be a very important consideration. Selye, *et al.* (1940) has stated that the adrenal hypertrophy seen as a result of

shock characteristically occurs during the second stage of shock ('counter-shock'). During the first phase one can readily imagine that, in addition to the increased protein destruction that is often caused by the shock-precipitating episode, the peripheral circulatory failure might result in very extensive tissue breakdown. The rapid rise in the non-protein nitrogen level in the blood (Taylor and Lewis, 1915; Aub and Wu, 1920) may be partially explained on this basis, for certainly it occurs too early to be due entirely to failure of renal function. Engel, Winton and Long, (1943) have recently demonstrated a striking rise in the concentration of amino nitrogen and keto acids in the blood of rats during hemorrhagic shock. These changes were associated with beginning depletion of the adrenal lipoid as early as one hour after hemorrhage. By 6 hours lipoid depletion was marked and in 24 hours a statistically significant increase in adrenal size was present. Other workers (Buell, 1919; Endres and Neuhaus, 1925) have reported increased nitrogen excretion after hemorrhage. The relationship between the changes in protein metabolism during shock and the histologic evidences of adrenal cortical activation are now under investigation in this laboratory.

Many clinical and experimental reports of adrenal hypertrophy following burns are available (Endres and Neuhaus, 1925; Kolisko, 1914; Pfeiffer, 1920; Niemeyer, 1921; Nakata, 1925). The theory of increased protein catabolism in adrenal hypertrophy can be invoked here on the basis of *a*) tissue destruction as a result of the burn, and *b*) shock frequently occurs in burned animals.

Infection

Both clinical and experimental data reveal that the adrenals are frequently greatly enlarged in animals with acute and chronic infections. Much of the clinical material will be covered by Sarason in his review (unpublished). Andersen (1935) found very large adrenals in rats which died of spontaneous infections of the lungs and middle ears, demonstrating the importance of excluding from consideration the effect of alterations in experimental conditions on adrenal size any animals which prove to have infections at autopsy. This response of the adrenal knows no species boundaries [human (Sarason, unpublished); rat (Andersen, 1935), pigeon (Riddle, *et al.*, 1924)] and the variety of infecting agents which produce adrenal enlargement is striking (Sarason, unpublished). That the offending organism does not necessarily have to be alive and actively invasive to produce adrenal enlargement is shown by the fact that many authors (for example, Deanesly, 1931) have described adrenal hypertrophy following the injection of suspensions of many kinds of killed micro-organisms. Two workers (Deanesly, 1931; Menten and Smith, 1936) have called attention to the diminution in lipoid content of the glands in animals with prolonged infections, suggesting that the glands were activated according to the histochemical schemes described by Dosne and Dalton (1941) and Sarason (1943). The obvious lack of specificity of this reaction immediately suggests that it is not the infection itself which produces the adrenal hypertrophy, but rather some metabolic change that occurs as a result of the infection.

The metabolic changes produced by infectious fevers in man have been reviewed by Peters and van Slyke (1931) who cite many instances in which nitrogen loss accompanied infections. They believe that protein destruction and nitrogen excretion in these circumstances are related to the intensity of the intoxication, rather than to the body temperature, and quote the work of Graham and Poulton (1912) in support of this view. These authors found that exposure in a steam bath sufficiently prolonged to raise the rectal temperature to 103 and 104° F. did not appreciably affect the rate of nitrogen excretion. The dissociation of excessive nitrogen excretion from fever is also indicated by the fact that the nitrogen excretion often remains high for several days after the fever has disappeared. The conclusion of Peters and Van Slyke is highly pertinent to the subject of this review: "There is, then, a definite tendency for nitrogen catabolism to increase in febrile infectious diseases, which has been well called *toxic destruction of protein*. This is not merely an expression, as it is in hyperthyroidism, of greater energy requirement, increased total metabolism. It is rather due to autolysis of tissue."

Toxins

Adrenal hypertrophy has been described following the administration of a variety of toxic substances that bear little structural resemblance to one another. Morphine (Benetatio, *et al.*, 1935; Deanesly, 1931; Fazekas, 1939), histamine (Noble and Collip, 1941), carbon tetrachloride (Higgins and Cragg, 1937; Higgins and Ingle, 1938), ammonium hydroxide (Fazekas, 1939), thallium, lead (Fazekas, 1939), atropine (Selye, 1936, 1937), formaldehyde (Selye, 1937; Selye and Collip, 1936), nicotine (Fazekas, 1939), phenol (Brebl, 1933), indole (Brebl, 1933), arsenic (Daniel, 1939), cyanide (Messerle, 1927) and peptone (Whitehead, 1932) have all been implicated as causes of adrenal enlargement, and, usually, no attempt is made to analyze the mechanisms involved in the hypertrophy beyond the observation that the administration of these noxious compounds to an animal constitutes a form of stress. As in the case of infections the great variety of toxins which produce an increase in adrenal size suggests very strongly that many of them may produce their effects through a common mechanism.

Although none of the reports concerning adrenal hypertrophy following toxin administration contain studies of protein metabolism, there is evidence that the tissues of the body tend to break down in the presence of many poisonous substances. A rise in the blood non-protein nitrogen has been reported following exposure of the tissues to chloroform (Inami, 1931), various drugs and foreign proteins (Krause, 1926), and carbon tetrachloride (Lanherr, 1935); in the last case, most of the rise was due to accumulation of amino acids. Glaubitz (1921) found evidence of protein destruction after poisoning by a number of substances, including carbon monoxide, lysol, oxalic acid, mercuric chloride, potassium bichromate and pantopon, but none after morphine intoxication. This is a provocative observation, inasmuch as morphine administration is known to result in a striking degree of adrenal hypertrophy, and adrenalectomized animals and patients with Addison's disease are extraordinarily susceptible to the action of opiates.

Two of the substances mentioned above should, perhaps, be mentioned separately. Histamine (as Rubin, Kennedy and Ralli, 1941, have pointed out in their review) has long been regarded as a compound that may be intimately concerned with the physiology of the adrenal cortex, although the nature of the relationship has never been very clear. Cyanide, as has been mentioned, produces a condition that is fundamentally indistinguishable from anoxia.

Dietary Factors

Salt content of the diet. Although the adrenal cortex plays an important rôle in the control of electrolyte metabolism, there is no convincing evidence that dietary changes in sodium, potassium, or chloride have a definite effect on adrenal size. Thus Ingle and Kendall (1938) were unable to demonstrate any effect of variations in dietary sodium or potassium on compensatory adrenal hypertrophy after unilateral adrenalectomy. Sarason (1943, *b*) noted no change after either high or low potassium diets. Swanson and Smith (1936), on the other hand, studying the effect of inorganic salt restriction of organ growth, do describe a small increase in adrenal growth over that of the controls during the first six weeks, but later shrinkage in adrenal size occurs.

Rats fed for 2 months on a diet deficient in magnesium have been shown to have considerable adrenal hypertrophy (Kleiber, *et al.*, 1941). This is associated with a corresponding degree of thyroid hypertrophy and a 25 per cent increase in oxygen consumption. Adrenal or thyroid hypertrophy were not noted in rats on a calcium deficient diet, although these animals exhibited an increased fasting catabolism (Kleiber, *et al.*, 1940).

Vitamin deficiencies. Experimental work on the effect of the vitamins and vitamin deficiencies on adrenal size are difficult to evaluate since much of the work was poorly controlled and lacks confirmation. Inanition controls were rarely used. Comparisons sometimes were made between animals on diets which differed otherwise than in their vitamin content, and we now know that such factors as the protein content of the diet may have a significant influence on adrenal size. Moreover, some of the observations were made at a time when the vitamins had only begun to be separated from one another, and the diets were probably deficient in an unknown number of factors.

In vitamin B deficiency a two to three-fold increase in adrenal size is described in pigeons (Beznak, 1923), rats (Beznak and Perjes, 1935, *a*; Lockwood and Hartman, 1933) and in dogs (Goodsell, 1941). Yeast prevented this increase in the pigeons, although the birds still showed signs of undernutrition. The adrenal hypertrophy of fasting birds, however, is not prevented by vitamin B_1. Sure (1938) reports that vitamin B_1 deficiency produces both adrenal and pituitary hypertrophy, but that on repeated depletions of this vitamin the pituitary hypertrophy disappears and that of the adrenal is reduced 20 to 50 per cent. Compensatory hypertrophy after unilateral adrenalectomy is greatly increased in B_1 deficient rats (Lockwood and Hartman, 1933). Beznak and Perjes (1935, *a*, *b*) have developed the interesting thesis that the adrenal hypertrophy after muscular exercise is due to a relative vitamin B deficiency since they can prevent it by giving 5 times the dose of B complex necessary for an animal at rest. Perjes (1937), however, later reported that this effect

could not be achieved by thiamin or flavin but could by the use of autoclaved brewer's yeast. The rôle of thiamin in protein and carbohydrate metabolism is poorly understood, although its action as a co-enzyme for several reaction involving carbohydrate intermediaries, such as pyruvic acid, is well recognized. Studying carbohydrate stores in thiamin deficient pigeons on different diets, Abderhalden and Wertheimer (1933) found that at the onset of paralytic symptoms there was uniformly an increase in liver glycogen and that this could be prevented by the administration of thiamin. A high level of liver glycogen was found only when symptoms of avitaminosis were present. In birds on high protein diets without vitamin B_1 liver glycogen values as high as 6.3 per cent were reported. Consistent with these results are those of Lavrov and Yarusova (1936, 1937, 1939) who noted a sharp increase in nitrogen excretion with the onset of polyneuritis in thiamin-deficient pigeons. This could be prevented by the administration of B_1 even though the birds continued to lose weight on this diet. Thiamin was found to have no effect on the nitrogen excretion during starvation. Increased protein catabolism and probably an enhanced rate of gluconeogenesis are thus also present in thiamin deficiency and may be related to the adrenal hypertrophy.

Vitamin C deficiency is reported to be associated with adrenal hypertrophy in rabbits (Giedosz, 1935) and in guinea pigs (Bessesen, 1923). In the latter a 29 per cent increase was reported in severe scurvy, but it should be noted that these animals showed a considerable weight loss and were not compared with inanition controls.

No consistent changes have been reported in vitamin A deficiency (Lockwood and Hartman, 1933; Giedosz, 1935; Nitzkewitch, 1934).

In vitamin D deficiency adrenal hypertrophy has been described (Jackson and Carelton, 1925; Hermann, 1940). Jackson and Carelton noted an increase of from 44 to 64 per cent in gland weight in slightly to severely rachitic rats. However, the diet used by these workers was rather high in protein and we have recently demonstrated adrenal hypertrophy on such diets (see below).

Biddulph and Meyer (1941) report that vitamin E deficiency in male rats caused an increase in adrenal weight which was intermediate between that of normal and castrates until 13 months, after which the glands became heavier than in the normals or castrates. At the latter stage muscular dystrophy developed. In female rats adrenal hypertrophy was not noted at any stage in the E deficiency. It would seem that in the male gonadal atrophy may have played a rôle in increasing adrenal size at first, while later tissue protein breakdown associated with the muscular dystrophy may have been the continuing stimulus. Recently these same workers (Biddulph and Meyer, 1942) have found an increase in thyroid size and a statistically significant increase in the basal metabolic rate of their vitamin E deficient male rats. This was apparently due to an iodine deficiency in the diet and was preventable by the addition of iodide to the diet. The increased thryoid activity is obviously another factor influencing adrenal size in these animals.

Protein content of the diet. Fahr (1912) reported that the size of the adrenals

doubled in rabbits fed a diet of milk and eggs. In a report to follow the authors will describe adrenal hypertrophy in rats fed a high protein diet.

Miscellaneous Factors

Tumors. Sarason (unpublished) has data on adrenal weights associated with clinical neoplasms. He believes that those cases in which malignant disease is associated with emaciation and cachexia are more likely to exhibit large adrenals than are ones in which there is little wasting. Ball and Samuels (1938) noted adrenal enlargement in rats bearing experimental tumors, and, in addition, pointed out the fact that when tumor-bearing rats were hypophysectomized the usual adrenal atrophy was present.

Cholesterol. Injection of cholesterol (Hasch and Hajdu, 1939) or high cholesterol diets (Aylward and Stott, 1937) are reported to produce adrenal hypertrophy. No obvious explanation is available for this phenomenon unless it be that the gland stores cholesterol as a precursor to cortical hormones. Aylward and Stott found a large increase in the content of cholesterol ester in rabbit adrenals after feeding high cholesterol diets.

Thymus-adrenal relationship. One phase of the problem of adrenal hypertrophy can be dismissed summarily. A reciprocal relationship between adrenal and thymus size has been reported (Selye, 1937) and, in addition, a few observers have described hypertrophy of the adrenals following thymectomy. The physiologic implications of these findings are anything but clear, since the function of the thymus is unknown.

Adrenal glands during fetal and early postnatal life. Fetal adrenal glands are unusually large and although the involution of the glands that occurs during the immediate postnatal period has been recognized for many years, the physiologic significance of this change is still unknown (Benner, 1940). Bruch and McCune (1936) in a study of the electrolyte metabolism of newborn infants, were unable to demonstrate biochemical changes of the sort associated with adrenal cortical insufficiency.

Goldzieher (1934) has emphasized the fact that a change in blood oxygen saturation occurs with the transition from intra-uterine to extra-uterine life, for the oxygen saturation of fetal blood is only 63 per cent as compared with 95 per cent in the maternal blood. He also states that, although one-third of the weight of the adrenals at birth is ordinarily lost within the first week of extra-uterine life, 3 infants with cardiac septal defects exhibited adrenal weights equal to those of newborn infants although the autopsies were performed at 21, 6 and 5 days, respectively. The author discusses the evidence for the increased dissociation of oxygen in fetal blood and notes the similarity between this phenomenon and the experimental findings at high altitudes.

Some workers have claimed that large adrenal glands are, in some way, related to sexual differentiation of the fetus, and that one of the functions of the fetal glands is to protect the fetus against an excessive influence of maternal estrogens by secreting large amounts of androgenic substance. This point of view is discussed by Windle (1940).

Animals thyroidectomized (Salmon, 1941) or hypophysectomized (Collip,

et al., 1933) during the immediate postnatal period do not exhibit the responses characteristic of those of older animals. From this one can infer that the metabolic regulatory mechanisms of the very young animal may be quite different from those that come into play later in the development of the individual. Therefore, the interpretation of the behavior of the adrenals during the first weeks of extra-uterine life in terms of what we know of adrenal physiology in the adult may be quite unjustified.

DISCUSSION

Since many of the conditions discussed in this review are characterized, in part, by increased protein catabolism, we have classified the well-authenticated conditions in which adrenal hypertrophy has been described in the following manner:

I. Primary increase in adrenotropic hormone
   Administration of adrenotropically active pituitary extracts
II. Secondary increase in adrenotropic hormone
   1. Compensatory hypertrophy
      A. After unilateral adrenalectomy or bilateral enucleation
      B. Parabiosis of an intact and adrenalectomized animal
      C. Vicarious action of fetal adrenals
   2. Estrogen treatment
   3. Absolute increase in cortical hormone requirement
      A. Increase in products of endogenous protein catabolism
         a. Secondary to hypermetabolism
            Thyroxin administration. cold, exercise, puerperium and lactation, magnesium deficiency
         b. Relative carbohydrate deprivation with obligatory increase in protein catabolism
            Starvation, phlorizin diabetes, insulin treatment
         c. Secondary to other causes
            Hemorrhagic and traumatic shock, burns, anoxia, infections, toxins, thiamin deficiency
      B. Increase in products of exogenous protein catabolism
         a. High protein diet

This classification is designed to show one point of similarity among the varied conditions previously discussed in detail. It is not intended to deny the possibility that other points of similarity may exist, and may therefore be related to the adrenal cortical hypertrophy that is common to these states. Nor should it be inferred that further investigation of individual 'stresses' will not reveal significant and pertinent biochemical differences between them which will make it necessary to postulate more than one mechanism for activation of the adrenal cortices. Until such differences are described, however, it would seem desirable to regard the adrenal hypertrophy seen in the conditions listed above as a manifestation of a fundamentally similar physiologic pattern.

The experiments on pregnant adrenalectomized animals and those on parabiotic twins strongly suggest that the primary stimulus for adrenal

hypertrophy is a humoral one, and, therefore, that the activation of the adrenals is the ultimate result of either a change in concentration of some material contained in the body fluids, or in some physico-chemical attribute of the fluids themselves. This change may affect adrenal size (and, presumably, hormone output) by means of its influence on the adrenocorticotropic activity of the anterior pituitary. For, on the basis of the facts herein presented, the adrenal glands appear to be stimulated to hypertrophy by the anterior hypophysis. The only possible exception to this rule is the case of certain cyclic variations in adrenal size noted in hypophysectomized, spayed rats in which artificial, threshold estrous cycles were maintained with estrogen (Bourne

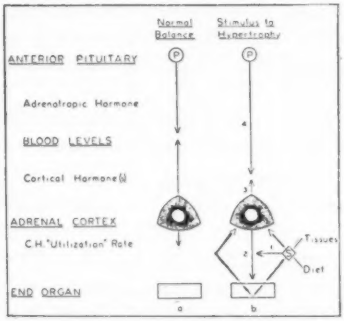

Fig. 1. Schematic outline of an hypothetical mechanism for adrenal hypertrophy. *a*. Normal balance between cortical hormone and adrenotropic hormone with normal rate of cortical hormone inactivation. *b*. Sequence of events (1–4) following introduction into the body of large amounts of substrate ('S') which requires cortical hormone for its metabolism. The dotted lines represent alternative routes by which S may stimulate the adrenal cortex to discharge its appropriate hormone.

and Zuckerman, 1940). From the work of Uotila (1940) it may be tentatively concluded that the stimulation of the anterior hypophysis is humoral in nature, and does not require the mediation of the central nervous system.

It is possible that a change produced by an increase in the rate of carbohydrate utilization might be a stimulus for adrenal hypertrophy. However, examination of the material presented above reveals that in all cases in which an acceleration of carbohydrate combustion can be postulated there is also an associated increased rate of protein breakdown, although the time relationships of these processes are not always clear. Moreover, excessively rapid protein catabolism is common to many more of the conditions we have discussed than is acceleration of carbohydrate oxidation. Ultimately, the solution of this problem depends on the determination of the site or sites of action of the adrenal cortical hormones.

We have been impressed by a rather sharp division between two different types of hypertrophy: *a*) that exemplified by such apparently unrelated conditions as shock, exercise, or high protein diet in which the sequence begins with an increase in the proportion of protein in the metabolic mixture, and

this is followed by adrenal enlargement; *b*) that typified by the hypertrophy of the adrenals associated with the administration of the natural and synthetic estrogens in which this sequence appears to be reversed. In addition to these types of hypertrophy there may be others which are manifestations of quite different biochemical processes. For example, hypertrophy by cholesterol may represent storage of hormone percursor, although this problem has not yet been investigated. Again, hypertrophy and hyperplasia of certain specialized cell types may occur either clinically or experimentally; diffuse bilateral androgenic hyperplasia of the cortex and the cortical hypertrophy with estrogen administration after ovariectomy described by Wooley *et al.* (1939) and by Gardner (1941) are examples of this.

A diagrammatic speculation on the possible mechanisms of adrenal hypertrophy is given in figure 1. It is intended merely as a graphic representation of a series of interrelated hypotheses. These may be summarized in the following manner.

The cortical hormone may inhibit the adrenotropic activity of the anterior lobe, and the adrenotropic secretion may therefore increase if the cortical hormone concentration in the body fluid falls. A fall in the cortical hormone concentration may be brought about by an increase in the rate of inactivation ('utilization') of the hormone. This may be a function of the hormone requirement of the organism. The hormone requirement may be increased by the presence in the body of excessive amounts of a substrate (S) which requires the presence of the hormone for its catabolism. It is possible to conceive of this substance as mobilizing cortical hormone either by way of the end organ in which it is catabolized, or by acting directly on the adrenal cortex. Finally, it is suggested that this substrate may be derived from the animal's own tissues (as in various 'stresses'), or from dietary protein.

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EFFECT OF HIGH PROTEIN DIETS ON SIZE AND ACTIVITY OF THE ADRENAL CORTEX IN THE ALBINO RAT[1]

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A REVIEW of the literature of adrenal hypertrophy (Tepperman, Engel and Long, 1943) revealed that many of the circumstances in which such hypertrophy occurs are characterized by either a relative or absolute increase in the rate of protein catabolism. This fact recurred so insistently that the reviewers have classified the 'causes' of adrenal hypertrophy according to the characteristics of their nitrogen economy.

On the basis of this suggestion, and in the light of the hypothetical chemical sites of action of the cortical steroids of the corticosterone type, experiments were devised to test the effect of high protein diets on adrenal size. This study was undertaken in an effort to determine whether or not evidence of increased adrenal cortical activity could be secured in animals which were forced to derive a large proportion of their carbohydrate from protein. In addition to morphologic evidence of increased cortical activity, an attempt has been made to compare the metabolic behavior of animals on a high protein diet with that of animals given physiologically active steroids. The work of Mirski, *et al.*, (1938) had already demonstrated how efficiently rats can become adapted to high protein diets by maintaining their carbohydrate stores during fasting, but these workers included no endocrine weights in their report.

MATERIALS AND METHODS

Male rats of the Sprague-Dawley strain were used, and all diets were fed *ad libitum*. The control diet consisted of Purina fox chow which (according to a calculation from the manufacturer's data for 'digestible' fiber-free and water-free material) contains 24.4 per cent of protein, 6 per cent of fat, 61 per cent of carbohydrate and 9 per cent of ash. The high protein diets consisted of the following: *a*) raw ground beef supplemented with cod liver oil and Osborne and Mendel's salt mixture (about 55 per cent of protein and 40 per cent of fat); *b*) casein A, consisting of casein 78 per cent, Mazola 5 per

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cent, brewers' yeast 10 per cent, salt mixture 5 per cent and cod liver oil 2 per cent; c) casein B, which contained casein 60 per cent, glucose 20 per cent, Mazola 4 per cent, yeast 10 per cent, salt mixture 5 per cent and cod liver oil 1 per cent. Animals on the ground-beef diet and casein A weighed 60 ± 2 gm. at the beginning of the experiment and were maintained on their respective diets for 4 to 7 weeks; their growth was at about the same rate as that of rats fed the control diet. The rats receiving the casein B diet, and which weighed 100 ± 2 gm. at the outset, were killed after 2 weeks on the high protein diet. All animals were fasted for 24 hours before they were killed. The adrenal glands were dissected free of fat, weighed on a micro-torsion balance and fixed in 10 per cent formalin. The glands were examined histologically by Dr. Ernest Sarason.

TABLE 1. COMPARISON OF ADRENAL WEIGHTS IN ANIMALS FED MEAT AND CHOW DIETS

	NUMBER OF ANIMALS	WEIGHT	ADRENAL WEIGHT (2)	ADRENAL WEIGHT / BODY WEIGHT
		gm.	mg.	gm./kg.
Controls	18	239 ± 12.1	36.7 ± 2.14	0.157 ± 0.00865
Meat-fed	18	227 ± 10.2	52.0 ± 3.32	0.236 ± 0.0115

Six animals, 100 to 120 gm. in weight, were subjected to adreno-demedullation under ether anesthesia, and the extent of regeneration of the cortex in the animals fed protein diets and in those fed carbohydrate diets was compared 4 to 5 weeks later.

Oxygen consuption was determined by the Haldane open circuit method. Glycogen was studied by means of the Somogyi modification of the Good and Kramer method (1933). Blood sugars were estimated by the method of Somogyi (1937). Blood pyruvate was measured according to the method of Lu, as modified by Bueding and Wortis (1940). Blood amino-acid nitrogen was determined by Frame's micro-modification of Folin's beta-naphthoquinone sulfonate colorimetric method (1943). Blood pyruvate and amino nitrogen studies were made one hour after the animals were anesthetized with 4 mg. of nembutal per 100 gm. of body weight.

The crude saline extract of beef anterior pituitary was prepared according to the method of Bonsnes and White (1940) and the casein hydrolysate containing tryptophane was prepared for us by Dr. Abraham White (1942). During the administration of anterior pituitary extract all animals received a high carbohydrate diet by stomach tube.

EXPERIMENTAL RESULTS

Rats fed a high protein diet showed marked hypertrophy of the adrenal glands. The results are given in table 1.

Histologically the enlarged glands are characterized by widening and lipoid depletion of the cortices (see fig. 1–4). The changes conform to the type described by Dosne and Dalton (1941) and by Sarason (1943), and have been interpreted as suggesting activation of the glands. This general microscopic picture is indistinguishable from that seen in acute anoxia, shock and intoxication with a variety of poisons.

Although there was no obvious difference in the size of the medullae of the two groups of glands, the extent of regeneration of enucleated glands in rats on high protein and high carbohydrate diets was tested. The results appear in table 2.

Four meat-fed and 3 chow-fed rats were given increasing amounts of crude saline extract of bovine anterior pituitary intraperitoneally for a period of 2 weeks. The adrenotropic activity of such extracts has been demonstrated by Bonsnes and White (1940). During the experimental period they received

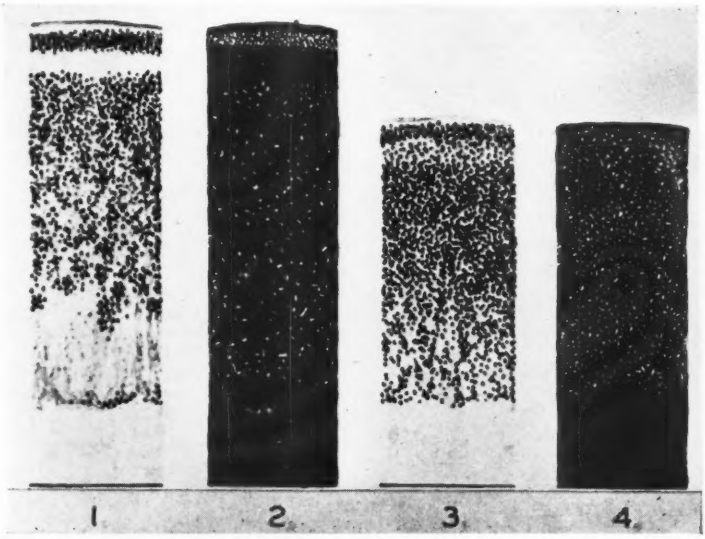

Fig. 1–4. Drawing of histologic appearance of adrenal glands obtained from meat-fed (1, 2) and chow-fed (3, 4) animals. Figures 1 and 3 are drawn from sections stained with Sudan stains; sudanophilic material is shown in black. Figures 2 and 4 are digitonin preparations in which the white areas represent digitonin-precipitated material. Note lipoid depletion in figures 1 and 2.

a high carbohydrate diet by stomach tube twice daily. All of the rats showed a mild glycosuria, and there was no difference in the amount of glucose excreted by the 2 groups. The weights of the paired adrenal glands are given in table 3.

Since adrenal cortical hypertrophy occurred in animals fed a diet of raw ground beef, certain observations were made on the metabolic characteristics of meat-fed animals. This phase of the problem was approached with two objectives in view: *a*) to discover the nature of the continuing stimulus to hypertrophy in the meat-fed animals, and *b*) to secure evidence concerning the level of activity of the hypertrophic adrenal cortices in this circumstance.

No statistically significant difference in oxygen consumption of the two groups of animals was found. Eighteen meat-fed animals consumed 145 ± 8 cc. of oxygen after a 24-hour fast, whereas the corresponding value for 18 chow-fed controls was 135 ± 7 cc. However, in the absence of 24-hour measure-

TABLE 2. EFFECT OF MEAT AND CHOW DIETS ON THE EXTENT OF REGENERATION OF ENUCLEATED ADRENAL GLANDS

RAT	DIET	WEIGHT	ADRENAL WEIGHT (2)	ADRENAL WEIGHT / BODY WEIGHT
		gm.	*mg.*	*gm./kg.*
DM H 4	Meat	312	50	0.160
DM H 1	Meat	298	40	0.134
DM H 2	Meat	284	45	0.158
DM C 1	Chow	242	29	0.120
DM C 3	Chow	250	23	0.092
DM C 4	Chow	248	22	0.089

ments of heat production, hypermetabolism cannot be ruled out as a factor in the production of adrenal hypertrophy in the meat-fed rats.

Blood keto acids (as pyruvate) and blood amino-acid nitrogen levels determined after a 24-hour fast revealed no definite differences between the two groups. The fluctuations of the blood amino-acid nitrogen following the subcutaneous administration of an hydrolysate of casein (0.12 gm. per 100 gm. of body weight) did not reveal any difference in the rate of disappearance of the injected amino acids in the groups on the two different diets.

TABLE 3. EFFECT OF ANTECEDENT DIET ON THE ADRENAL HYPERTROPHY PRODUCED BY CRUDE SALINE EXTRACT OF ANTERIOR PITUITARY

RAT	ANTECEDENT DIET	WEIGHT	ADRENAL WEIGHT (2)	ADRENAL WEIGHT / BODY WEIGHT
		gm.	*mg.*	*gm./kg.*
C 6	Chow	266	75	0.282
C 8	Chow	274	75	0.274
C 13	Chow	266	67	0.252
H 9	Meat	296	104	0.349
H 11	Meat	272	114	0.419
H 12	Meat	285	91	0.320
H 21	Meat	310	90	0.294

However, the behavior of the blood glucose following the administration of casein hydrolysate was strikingly different in the 2 groups. The anesthetized meat-fed animals uniformly showed a hyperglycemia reaching 180 mg. per cent, whereas the blood sugar of identically treated control rats did not change appreciably over a period of 4 hours after the parenteral administration of amino acids. The mechanism of this phenomenon became apparent when the experiment was repeated on adreno-demedullated animals; it was then found that neither meat-fed nor chow-fed rats exhibited a rise in blood glucose after subcutaneous injection with casein hydrolysate. Consequently, the hyperglycemia observed was due to the effect of the amino acids on the activity of the adrenal medulla, as has already been noted by Basiliou and Zell (1931).

A comparison of the fasting carbohydrate levels of meat-fed and chow-fed rats revealed significantly higher values in the former animals. Figure 5 is a graphic representation of the blood sugar and liver glycogen of animals on

chow and on ground beef diets. The behavior of the carbohydrate levels in chow-fed rats injected with adrenal cortical extract and Kendall's compound E are given for comparison. It will be seen that, in agreement with the work of Mirski and his colleagues (1938), the changes in the meat-fed animals are in the same direction as those produced by the injection of cortical extract and compound E. While this is by no means conclusive evidence, it suggests that the enlarged adrenal cortices of the meat-fed animals may secrete unusually large amounts of hormone. This is one instance, then, in which cortical enlargement is associated with lipoid depletion of the cortex, and, very

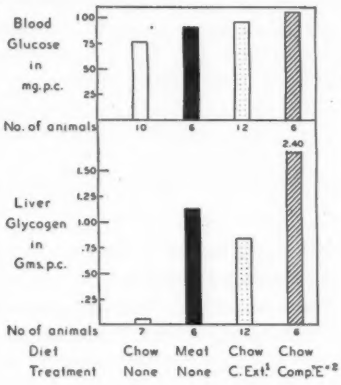

Fig. 5. Comparison of liver glycogen and blood glucose levels in chow-fed and meat-fed animals after a 24-hour fast. The corresponding levels for chow-fed animals treated with cortical extract and Kendall's compound E are also shown.

[1] Data of Long, Katzin and Fry.
[2] Data of Long and Fry.

likely, with an increased rate of secretion of the corticosterone type of hormone.

In view of the fact that the ground meat diet proved to contain about 40 per cent of ether-extractable material, a synthetic high protein diet (casein A) containing 7 per cent of fat and 78 per cent of casein was tested for its effect on adrenal size. The results of this experiment are given in table 4.

TABLE 4. EFFECT ON ADRENAL SIZE OF A SYNTHETIC HIGH PROTEIN DIET

DIET	NUMBER OF ANIMALS	WEIGHT	ADRENAL WEIGHT / BODY WEIGHT
		gm.	gm./kg.
Chow	6	221±5	0.139±0.004
Casein A	5	216±4	0.195±0.012

Both the meat diet and the synthetic high protein diet (casein A) were practically free of carbohydrate. It was conceivable, therefore, that the observed effects were not due to a positive effect of excessive dietary protein,

but were a consequence of carbohydrate deprivation. This possibility required investigation inasmuch as one effect of the 'protein-carbohydrate' type of adrenal cortical steroid is inhibition of carbohydrate oxidation (Ingle, 1941; Evans, 1941). A diet (called casein B) was therefore devised in which one quarter of the protein in the casein A diet was replaced by an equivalent amount of glucose. It was found that animals fed this diet for 2 weeks showed marked adrenal hypertrophy; details of the experiment are given in table 5.

TABLE 5. EFFECT ON ADRENAL SIZE OF A HIGH PROTEIN DIET CONTAINING 20 PER CENT OF GLUCOSE

DIET	NUMBER OF ANIMALS	WEIGHT	ADRENAL WEIGHT / BODY WEIGHT
		gm.	*gm./kg.*
Chow	10	191±3.2	0.158±0.003
Casein B	11	180±4.4	0.226±0.0088

We are grateful to Dr. Ernest Sarason for examining the histologic preparations and to Mr. Armin Hemberger for figures 1 to 4.

The discrepancy in body weight between the two diet groups is believed to be due to the fact that the animals getting the casein B diet were autopsied 5 days before their controls were killed. From this experiment it may be concluded that the ingestion by rats of a diet containing protein and glucose in the ratio of 3:1 results in about a 45 per cent increase in weight of the whole adrenal gland in 2 weeks. Since the meat diet produced a 50 per cent hypertrophy and the casein A diet a 40 per cent increase in adrenal size, it is apparent that the enlargement of the glands in the casein B experiment was not inhibited by the presence of glucose in the diet.

These results are in good agreement with those of Fahr described in 1912. This investigator reported doubling in the size of adrenals of rabbits fed a diet of milk and eggs. Fahr, who made this observation during the course of a study of experimental atherosclerosis, believed that the adrenal hypertrophy was a degenerative phenomenon.

Although Limson and Jackson (1932) reported no change in adrenal weight of young rats maintained on a low protein diet, analysis of Orten's data (1937) on endocrine weights supplements the material presented here. This investigator found the adrenal weight body weight ratio to be the same (0.202 gm./kg) in markedly stunted female rats fed on a 3.5 per cent protein diet as in rats fed an 18 per cent protein diet *ad libitum*. Rats consuming the same number of calories as those on the 3.5 per cent protein diet, but with as much protein as eaten by the controls on the 18 per cent protein diet, showed a marked adrenal hypertrophy (adrenal wt./body wt., 0.445 gm./kg). If, however, the protein was kept at 3.5 per cent and the calories made up by force-feeding with carbohydrate and fat, no adrenal hypertrophy followed. Thus, the protein content of the diet again is seen to be a determining factor in adrenal size.

SUMMARY

Studies have been made of the effect of variations in the protein and carbohydrate content of the diet on the adrenal size of albino rats. Animals fed raw ground beef (55% protein) or a 78 per cent casein diet for 4 to 7 weeks showed a 40 to 50 per cent increase in adrenal size when compared to control rats fed Purina fox chow (24.4% protein, 61% carbohydrate). Hypertrophy of the enucleated glands of rats on a high protein diet demonstrated that the enlargement was primarily cortical. A similar degree of hypertrophy was observed after the feeding of a diet containing 60 per cent casein and 20 per cent carbohydrate demonstrating that the low carbohydrate content of the previous diets probably was not the cause of the enlargement. The cortical cells of the enlarged glands showed lipoid depletion on histologic examination. Saline extract of the anterior pituitary greatly increased the gland size of both chow and meat-fed animals over that achieved by diet alone.

Examination of the carbohydrate levels in the two groups revealed elevations of the fasting blood sugar and liver glycogen in the meat-fed rats comparable to that seen after administration of potent cortical extracts. There were no significant differences in the oxygen consumption or blood keto and amino-acid levels in the two groups. The rats fed the high protein diet exhibited a striking rise in blood sugar after the parenteral administration of amino acids, but this effect was abolished by adreno-demedullation.

On the basis of the survey of the literature and the experimental data on animals receiving high protein diets it is suggested that some event or series of events occurring during the catabolism of protein may serve as a stimulus for the hypertrophy of the adrenal cortex observed in a variety of circumstances.

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EFFECT OF DIET IN RATS ON ADRENAL WEIGHTS AND ON SURVIVAL FOLLOWING ADRENALECTOMY

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ONE OF THE functions of the adrenal cortex is concerned with the formation of carbohydrate from protein and with the maintenance of normal stores of carbohydrate in the body (Long, *et al.*, 1940). It was, therefore considered possible that the size and functional activity of the adrenal cortices in normal animals and the periods of survival of adrenalectomized animals might be influenced by the proportions of carbohydrate and protein in the diet. The results of the present study fail, however, to support this hypothesis.

METHODS

Male rats of the Sprague-Dawley strain were used. They were fed a diet of Purina dog chow until they reached a weight of 180 to 182 gm. They were

TABLE 1. COMPOSITION OF VARIOUS DIETS EMPLOYED

CONSTITUENT	HIGH CARBOHYDRATE	HIGH PROTEIN	HIGH FAT	MIXED
	gm.	gm.	gm.	gm.
Dried yeast (Pabst)	100	100	100	100
Salt mixture (Osborne & Mendel)	40	40	40	40
Wheat germ oil	10	10	10	10
Cod liver oil	10	10	10	10
Cellu flour (Chicago Diatetic Supply)	120	120	120	120
Mazola oil	10	10	10	175
Whole milk powder (Merrell-Soule)				600
Casein (Labco)	160	830	160	
Dextrin	460			
Starch	500			
Butter fat		140	440	

then maintained on the diets fed *ad libitum* (table 1). The proportion of the total calories in each diet which are derived from protein, carbohydrate and fat are given as percentages in table 2.

After periods of 7, 14 and 28 days the rats were adrenalectomized by a procedure previously described (Ingle and Griffith, 1942). The excised adrenals were cleared of fat and connective tissue and weighed under cover. Daily body weights and the periods of survival were recorded.

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In order to obtain data indicating the extent of adrenal cortical hypertrophy under stress, 20 normal male rats weighing 180 gm. each and which had been maintained on dog chow, were anesthetized with sodium phenobarbital and were subjected to continuous stimulation of the gastrocnemius muscle for a period of 120 hours (Ingle and Lukens, 1941). At the end of this time the animals were killed and the adrenal glands were removed and weighed. In addition, 35 normal rats weighing 180 gm. each and which had been maintained on dog chow were killed and the adrenals weighed.

EXPERIMENTS AND RESULTS

Normal rats were equally divided among the four different diet groups so that 24 were maintained for a period of 7 days and then adrenalectomized, 28 for a period of 14 days and then adrenalectomized, and 28 for a period of 28 days and then adrenalectomized. Following adrenalectomy these animals

TABLE 2. PROPORTION OF TOTAL CALORIES DERIVED FROM CARBOHYDRATE, PROTEIN AND FAT IN DIETS DESCRIBED IN TABLE 1

CALORIC SOURCE	DIET			
	HIGH CARBOHYDRATE	HIGH PROTEIN	HIGH FAT	MIXED
Carbohydrate	77.6	2.3	2.3	20.1
Protein	16.2	67.5	16.2	16.3
Fat	6.2	30.2	81.5	63.6

were maintained on the same diets and on tap water throughout the subsequent survival period. In addition, 24 normal rats were equally divided among the four diet groups and were maintained for a period of 7 days and then adrenalectomized. Following removal of the adrenal glands, these animals were maintained on the same diets and on a 1.0 per cent sodium chloride solution for a period of 6 weeks. The animals were then given water to drink for the remainder of the survival periods.

Adrenal-weight. The data on adrenal weights are summarized in figure 1. There were small but probably insignificant differences in the average weights of adrenals of rats which were fed a mixed, high carbohydrate or high protein diet. There was some tendency for the rats maintained on a high fat diet to have adrenals which were heavier than normal. Whether this greater size was due to an increase in the number or size of individual cells or to an increase in the storage of adrenal fat was not determined. The rapid and extensive hypertrophy which characterizes the adrenal glands of rats subjected to stress is illustrated by the adrenal weights of the rats subjected to continuous work of the gastrocnemius muscle.

Survival. The data on survival of the rats following adrenalectomy are shown in figure 2. All of the untreated rats died within 7 to 18 days following operation. There was no significant relation of the period of survival to the content of carbohydrate, protein or fat in the diet. On the other hand, all of the rats which were given 1.0 per cent sodium chloride solution to drink

following operation survived and gained in weight until the sodium chloride solution was withdrawn and replaced by water. All of the rats then died within a period of 12 days and again there was no relation of the survival periods to the carbohydrate, protein or fat content of the diet.

Weight. It is shown in figure 3 that all of the rats gained in weight prior to adrenalectomy and that the extent of these gains was not significantly

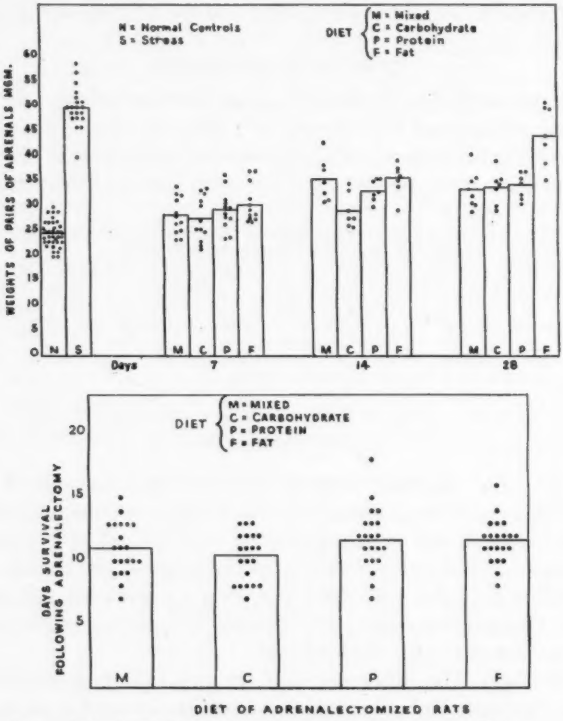


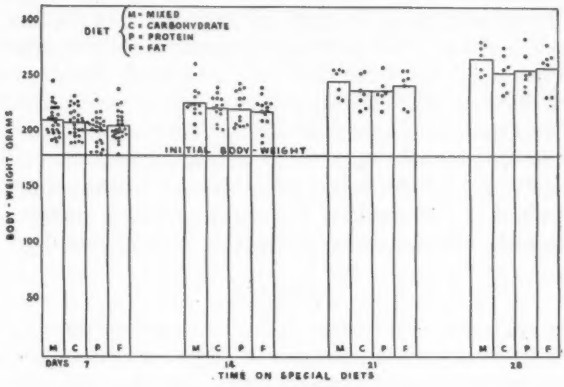
Fig. 1. (*upper*) Weights of adrenal glands of rats fed different diets.
Fig. 2. (*lower*) Survival time of adrenalectomzed rats fed different diets.

related to the composition of the diet. Following adrenalectomy these rats lost from 16 to 61 gm. within the first week and the extent of these losses was not significantly related to the nature of the diet. It is shown in figure 4 that the rats which were maintained on 1.0 per cent sodium chloride gained in weight until the sodium chloride was withdrawn. The greatest gains were shown by the rats which were maintained on the high protein diet whereas the rats given the high fat diet gained more slowly than the others.

DISCUSSION

The adrenal cortex of the rat undergoes atrophy when excessive amounts of the cortical hormones are administered (Ingle, *et al.*, 1938) and hypertrophies when the animal is subjected to stress. If these wide changes in weight are

indirectly determined by the requirements of the organism for carbohydrate it might be expected that the adrenal cortices would atrophy when excessive amounts of exogenous carbohydrate are available and would hypertrophy when it is necessary to form most of its carbohydrate from protein. The results of this study do not support the hypothesis nor can it be said that they



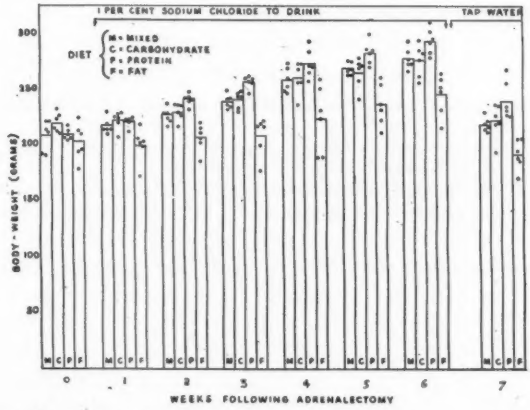


Fig. 3. (*upper*) Body weights of normal rats fed different diets.
Fig. 4. (*lower*) Body weights of adrenalectomized rats fed different diets and given saline to drink.

constitute disproof. The adrenal glands of the rats fed the high protein diet did show a slightly greater average weight than the adrenal glands of rats fed the high carbohydrate diet. These small differences may have been due to chance or chance variation may have obscured a greater true difference. It is not definitely known whether the weight of the adrenal gland is a reliable index of the secretory activity of the cortex. Also, the requirements for carbohydrate may be much greater in animals which are subjected to stress than in animals during the resting conditions which characterize this experiment. However, it is possible that cortical hypertrophy during stress does reflect an increased secretion of cortical hormones to meet a physiologic need by the

organism for the control of some unidentified function or functions not closely related to the availability of carbohydrate.

There is little evidence that the amount of carbohydrate in the diet is related to the survival time of untreated adrenalectomized animals. The importance of the defect in carbohydrate metabolism which characterizes adrenal cortical insufficiency is likely to be manifest only during fasting. The results of the present study illustrate the importance of the level of dietary intake of sodium chloride but were negative as far as the proportions of carbohydrate, protein and fat of the diet were concerned. Richter (1941) found that the self-selected diet of the adrenalectomized rat is low in carbohydrate. In unpublished studies we have observed that adrenalectomized rats force-fed a diet high in carbohydrate survived for shorter periods than did rats force-fed an isocaloric amount of a mixed diet. However, it was not possible to dissociate the effect of carbohydrate *per se* from possible artifacts due to the greater bulk and osmotic activity of the high carbohydrate diet.

SUMMARY

A study was made of the effect of different proportions of carbohydrate, protein and fat in diet on the weights of adrenal glands of rats and on the periods of survival following adrenalectomy. The observed differences were small and probably without significance.

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OBSERVATIONS ON THE RELATIONSHIP OF THE THYROID TO THE POLYURIA OF EXPERIMENTAL DIABETES INSIPIDUS

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THE FEEDING of large doses of desiccated thyroid to animals with diabetes insipidus is known to produce an exaggeration of the polyuria already present (Fisher and Ingram, 1936; White, *et al.*, 1938; Bruhn, 1939; Hare, 1940; Clarke, 1940). Conversely, thyroidectomy reduces the urine volume in diabetes insipidus (Fisher and Ingram, 1936; Blotner and Cutler 1941; Hare, 1940). In animal experiments the reduction of the polyuria amounted to as much as 25 to 50 per cent, but the urine volume under these conditions was still 2 to 4 times the volume in normal cats.

The specific rôle of the thyroid gland in the maintenance of the full polyuria of diabetes insipidus is not known. No studies of renal physiology in such animals after thyroidectomy have been reported. The diuretic effect of thyroid feeding appears to be associated with the metabolism-stimulating factor (Bruhn, 1939), although the administration of such substances as dinitrophenol and dinitroorthocresol has no effect on water exchange in animals with diabetes insipidus (White, *et al.*, 1938; Clarke, 1940). The experiments of Brull (1940) in kidney transplantation indicate that the action of the thyroid substance in water exchange depends upon a direct renal effect. Hare (1940) found that the enhanced polyuria produced by feeding thyroid to a thyroidectomized dog with diabetes insipidus was associated with an increased glomerular filtration rate as indicated by creatinine clearance.

No observations have been made on the effect of thyroid feeding upon the excretion of sodium chloride in animals with diabetes insipidus. The need for such studies becomes apparent when it is recalled that feeding high salt diets to such animals causes a marked rise in water exchange (Fisher and Ingram, 1936; Winter, *et al.*, 1940). If thyroid feeding should cause an increased salt excretion in diabetes insipidus a possible factor in its diuretic action may be revealed. This communication deals with such experiments, as well as with observations upon chloride excretion after thyroidectomy.

It should also be pointed out that when cats with diabetes insipidus are placed on a diet of low nitrogen content the polyuria is reduced (Winter and Ingram, 1941). Furthermore, an increase in the nitrogen content of the diet is reflected by an increased urine output, the curve of which is strikingly parallel with that of nitrogen excretion. It is therefore logical that the effect

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of thyroid feeding upon nitrogen excretion and the relationship of the latter to the rise in urine volume should be studied. Such experiments will also be reported here.

MATERIAL AND METHODS

Diabetes insipidus was produced in cats by interruption of the supra-optico-hypophyseal tracts by electrolytic lesions in the hypothalamus or by direct section of the upper end of the infundibular stem. All of the animals with diabetes insipidus had well established polyuria as evidenced by daily records of water exchange for 2 to 12 months preceding our studies.

The cats were maintained on carefully measured diets, of which there were two: *Diet A* consisted of 80 gm. of raw ground lean beef and 100 cc. of whole milk and contained an average of 0.238 gm. of NaCl per feeding. *Diet B* consisted of 100 gm. of 'Red Heart A' and 100 cc. of whole milk and contained 0.657 gm. of NaCl. The animals were fed the entire day's ration in the same hour each morning after the daily urine collection. Thyroid preparations were mixed with the meat.

Three types of thyroid were used: thyroxin, Squibb; desiccated thyroid U.S.P., Cudahy, 0.22 per cent iodine content; desiccated thyroid, Cudahy, 0.64 per cent iodine.[1] Thyroxin was administered orally in daily doses of 0.8 mg.; this was usually increased after a few days to 1.6 mg. Thyroxin is known to be effective to a variable degree when given by mouth. Desiccated thyroid was fed in daily doses of 1 gm., or the approximate equivalent of 0.25 to 0.4 gm. per kg. (cf. Kunde, 1923).

Urine chloride was determined by a modified Volhard-Harvey technique after digestion of aliquots of the specimen. Urine nitrogen was estimated by a microkjeldahl procedure with Nesslerization and reading in a photo-electric colorimeter.

When high salt diets were used, 1.5 gm. of additional NaCl was divided between meat and milk and 0.5 per cent NaCl solution was substituted for drinking water.

RESULTS

Effect of thyroid feeding. Four cats with diabetes insipidus and 2 normal cats were fed the various thyroid preparations. Thyroxin was fed the operated cats in doses of 0.8 mg. for 4 days, immediately followed by doses of 1.6 mg. The Cl output appeared to increase somewhat during administration of the 0.8 mg. doses, but had begun to fall by the time the dose was increased to 1.6 mg. The chloride balance then definitely shifted so that a slightly positive balance resulted and was maintained for 10 days after cessation of thyroxin administration. The water balance showed no actual change, although there was a slight increase in urine volume. The normal cats received doses of 1.6 mg. of thyroxin for 7 days. The NaCl balance also shifted somewhat toward the positive side except for one day at the end of thyroxin feeding. There was no appreciable increase in urine output in the normal cats. It may

[1] Acknowledgment and thanks are due Mr. I. J. Klingaman of the Pharmaceutical department, the Cudahy Packing Co., for generous supplies of desiccated thyroid.

be said that thyroxin administered orally in the doses mentioned had but little effect in increasing urine volume, even in cats with diabetes insipidus.

After the chloride balance had returned to its previous consistent level (a period of 18 days), desiccated thyroid (0.22% I_2) feeding was instituted. Each animal received 1 gm. daily for 9 days. In both the operated and normal cats there was a slightly greater positive shift in the NaCl balance than with thyroxin feeding. This was not at first apparent; indeed, during the first part of the period of thyroid feeding the NaCl excretion increased somewhat so that there was a tendency to a negative balance, but toward the end of the feeding period the balance was definitely positive and usually remained so for at least a week after thyroid feeding had been discontinued. Urine output in the normal animals was affected but slightly, if at all. The experimental cats,

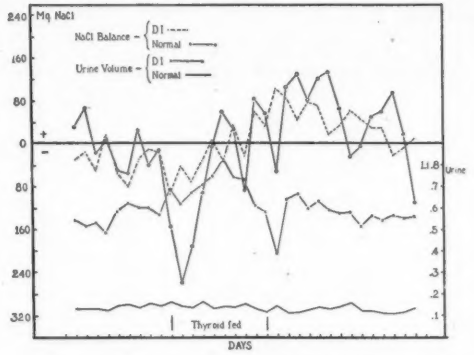


Fig. 1. Average daily balance of sodium chloride and the average daily urine volume of 4 cats with diabetes insipidus and 2 normal cats before, during and after a period of feeding desiccated thyroid (0.64% I_2).

however, showed marked elevation of the water exchange in every instance. Some of the average increases in urine output during the actual period of feeding (not considering the occasional persistent after-effect of thyroid feeding) amounted to 18, 29 and 21 per cent, while on certain days the percentage of increase reached 50, 74 and 43 per cent. In the operated cats the urine output reached its peak toward the end of the period of thyroid feeding. At this time of greatest diuresis the daily chloride output was diminished, so that a positive chloride balance prevailed.

Feeding of thyroid of higher iodine content (0.64% I_2) gave similar results (fig. 1). In the cats with diabetes insipidus, as the urine volume increased, the Cl balance became more positive, that is, there was a progressive decrease in Cl output. In the normal cats the Cl output increased during the first days of thyroid feeding and then rapidly declined so that the balance became positive. It is of interest that during the period of increased Cl excretion in the normal animals there was no increase in urine volume. The shifts in Cl balance were more marked under this treatment than under either thyroxin or the other type of thyroid substance.

In additional experiments on thyroid feeding, while the animals were on a high salt diet there was also a slight retention of chloride.

Average figures for urine volume and NaCl output under the various treatments for individual cats are given in table 1. The positive tendency of the Cl balance is not always discernible from these figures because of the rather delayed effects of thyroid feeding. When daily figures are plotted on a graph, however, the shift becomes evident, as in figure 1.

Effect of thyroidectomy and thyroid feeding. The thyroids were removed from 2 normal cats and 4 cats with diabetes insipidus. In general there was little change in chloride excretion, except in some cases there was a transient decrease in output, perhaps due to diminished metabolic activity. In the

TABLE 1. URINE AND CHLORIDE EXCRETION IN CATS WITH DIABETES INSIPIDUS AND IN NORMAL CATS

CAT	THYROXINE			0.22% I_2 THYROID			0.61% I_2 THYROID		
	5 DAYS CONTROL	10 DAYS FEEDING	10 DAYS POST-FEED.	9 DAYS CONTROL	9 DAYS FEEDING	16 DAYS POST-FEED.	10 DAYS CONTROL	9 DAYS FEEDING	10 DAYS POST-FEED.
1	465	534	533	480	617	516	436	729	542
	.309	.240	.269	.235	.252	.263	.279	.273	.218
2	759	796	775	804	949	790	735	855	750
	.274	.253	.212	.236	.268	.237	.276	.234	.224
3	465	417	465	489	513	453	481	291	379
	.225	.229	.174	.207	.236	.170	.254	.117	.109
4	650	772	680	665	803	709	637	866	625
	.271	.294	.235	.254	.292	.249	.282	.286	.198
A	141	139	117	115	102	114	138	130	110
	.316	.251	.215	.237	.239	.249	.273	.251	.152
B	180	139	125	122	142	124	145	148	154
	.252	.285	.213	.227	.269	.206	.263	.296	.186

Upper figures indicate the average daily urine output in cc. for the individual cats. The lower (decimal) figures are the average daily NaCl output in gm. Cats 1, 2, 3, 4 had diabetes insipidus; A and B were normals.

animals with diabetes insipidus the urine volume diminished. During the first part of this period there was a decline in chloride excretion lasting a few days (fig. 2). However, the chloride excretion soon returned to normal whereas the urine volume remained at the lower level. The cats gained weight during this time.

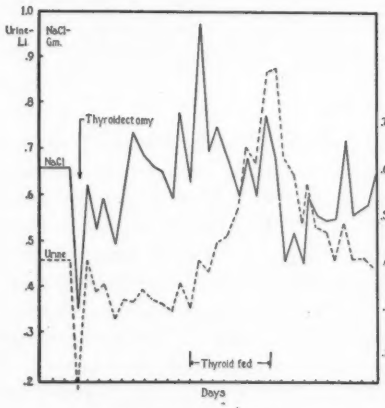
When the desiccated thyroid was fed in 1 gm. daily doses to the thyroidectomized cats there was a transient increase in chloride excretion of a few days' duration, followed by a reduction to normal or subnormal levels during the last days of the 7-day feeding period. This decline in chloride output occurred while the urine output of the cats with diabetes insipidus was rising to levels considerably above the normal (fig. 2, *cat 8*). Immediately after cessation of thyroid feeding the chloride excretion had decreased to such an extent that the daily chloride balance was decidedly positive. During this time the urine volume, although declining, was still far above normal. Then as the urine volume decreased further and approached normal levels, the

TABLE 2. NITROGEN PARTITION IN THYROID FEEDING; AVERAGE DAILY OUTPUT

CAT		TOTAL N	UREA N	AMMONIA N	URIC ACID N	CREATININE N	UNDETERMINED N
		gm.	gm.	gm.	gm.	gm.	gm.
5	Control period	2.64	2.31	.18	.003	.045	.10
	Thyroid feeding	3.07	2.62	.33	no data	.057	.06
6	Control period	2.56	2.18	.10	.002	.040	.24
	Thyroid feeding	3.14	2.72	.24	no data	.048	.13
C	Control period	2.69	2.22	.13	.003	.049	.29
	Thyroid feeding	3.18	2.63	.12	no data	.060	.37

chloride output gradually rose again. During and after the thyroid feeding the animals lost weight so that a week after discontinuing thyroid administration the weight loss aggregated nearly 0.5 kg. per animal. The period of maximum rate of weight loss coincided with the period of greatest diuresis and extended into that of greatest chloride retention.

Thyroid feeding, diuresis and nitrogen excretion. Winter and Ingram (1941, and unpublished data) have observed that alterations in the excretion of



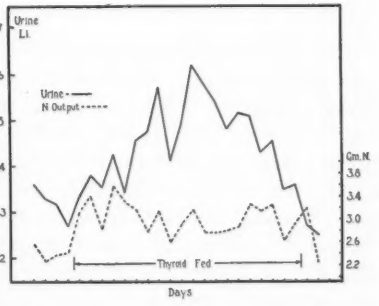


Fig. 2. (*left*) Effects of thyroidectomy and later thyroid feeding on daily NaCl excretion and urine volume in *cat 8*, diabetes insipidus.

Fig. 3. (*right*) Daily urine volume and total nitrogen excretion in *cat 6*, diabetes insipidus.[1]

nitrogen in the urine may be associated with changes in the level of polyuria in cats with diabetes insipidus. Thus, on a diet of standard caloric value and NaCl content, but low in nitrogen, the polyuria declines. On the other hand, a diet meeting the same standard requirement but high in nitrogen produces a definite increase in the polyuria. The added nitrogen need not be in the form of protein, since urea feeding elicits the same response. Variation of the daily excretion of nitrogen parallels changes in urine volume under the above conditions. Since it is generally held that thyroid feeding accelerates the metabolism of protein, it is not illogical to surmise that increased nitrogen excretion

might be associated with the elevated urine volume. The fact that cats with diabetes insipidus do not readily concentrate the nitrogenous constituents of the urine, while normal cats do, might enter into a future explanation of the observation that thyroid diuresis is easily produced in cats with diabetes insipidus but not in normal cats.

Two operated cats (5 and 6) and one normal one (C) were maintained on a standard diet containing 3.2 gm. of N daily, and the urinary N determined each day. The usual 1 gm. doses of desiccated thyroid (0.22% I_2) were fed for periods of 20 and 10 days, the added organic material bringing the N in the diet to 3.336 gm. The average results are shown in table 2 and the specific case of *cat* 6 is presented graphically in figure 3. In all 3 animals there was a rise in the daily nitrogen excretion during thyroid feeding amounting to a net average increase of about 12.5 per cent, allowing for the nitrogen in the thyroid substance and using a digestibility factor of 0.82. The increase was about the same for all 3 animals and there appeared to be no essential differences in the nitrogen partition. All of the nitrogen fractions were increased, the greatest increase occurring in the urea fraction. The urine volume was increased in the cats with diabetes insipidus. The increases in urine volume and in N output paralleled one another quite closely during the first 5 days, but after that the N output levelled off or fell slightly while the urine volume continued to increase for several days more. Thus only part of the thyroid diuresis might be accounted for by the elevated excretion of nitrogen.

DISCUSSION

It is evident from the above that the diuresis caused by feeding thyroid to cats with diabetes insipidus is not associated with a persistently increased chloride excretion. Such an increase may appear for a short time after initiation of thyroid feeding, but the chloride output falls below normal levels as diuresis develops, and small but definite amounts of Cl are retained. It is evident that diuresis occurs in spite of salt retention. It may perhaps be asked if this retained chloride affects in any way the polyuria or the superimposed diuresis. On the surface, no effect appears likely, since the amounts retained are so small. There is also no evidence from our data that the retention of salt has directly influenced thirst in our animals. An interesting corollary to the above observations is provided by findings made in this laboratory which have shown that a marked retention of chloride accompanies the enormous diuresis which is produced in cats with diabetes insipidus by administration of dilute salt solution (0.5% NaCl) in place of water. It may be mentioned that Shannon (1942) observed a retention of sodium under high salt intake in dogs with diabetes insipidus. While our data include no direct measurements of renal activity, his experiments indicated that the retained salt produced an expanded volume of extracellular fluid which in turn was presumed to cause an observed increase in rate of glomerular filtration. This increased glomerular filtration, in the presence of the diminished tubular reabsorption of water characteristic of diabetes insipidus, would be the main factor in saline diuresis, according to Shannon. It will be recalled that Hare found increased glomerular

filtration in dogs with diabetes insipidus which were fed thyroid substance. There is as yet no evidence, however, that this increase is due to the small amounts of salt retained under these conditions. In normal animals a thyroid diuresis does not appear, possibly because the effect of increased glomerular filtration would be compensated for by increased tubular reabsorption in the presence of the posterior pituitary hormone.

It has been indicated that the increased nitrogen excretion under thyroid feeding is not sufficient to account for all of the diuresis in the experimental cats, since the nitrogen excretion curve becomes level while the curve of urine volume is still rising. Whether the extra nitrogen available for excretion contributes to the elevated rate of glomerular filtration in these animals remains to be determined. However, it may be mentioned that Pitts (1935) and Hiatt and Hiatt (1942) have found that an increase in the exogenous nitrogen in dogs and seals is related to enhanced glomerular filtration.

SUMMARY

In cats with well established diabetes insipidus due to lesions of the hypothalamic-hypophyseal system, thyroid feeding produces an increased urine output which is associated with a positive shift in the chloride balance, *i.e.*, a diminished chloride output, although a brief period of increased chloride excretion may follow the initiation of feeding. In normal animals the same influence on chloride excretion is observed, but there is no significant diuretic response.

After thyroidectomy, the animals with diabetes insipidus had average volumes of urine which were 25 to 35 per cent less than during the previous period of polyuria, but these were still 3 to 4 times greater than the output of normal cats. The chloride excretion was affected but little after the immediate post-operative period of 1 or 2 days. Feeding thyroid to these animals had the usual effect as indicated above.

Associated with thyroid feeding there is a net rise in the daily excretion of urinary nitrogen of about 12 per cent in both normal cats and those with diabetes insipidus. The nitrogen excretion curve, however, does not parallel the curve of diuresis and the increased nitrogen excretion is probably only contributory to the mechanisms involved in the diuresis.

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OVARIAN AND PLACENTAL FUNCTION IN ADDISON'S DISEASE[1,2]

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THE RELATION of the adrenals to gonadal function has never been clearly elucidated (Looney, 1940). In the rat the removal of the adrenal gland has been reported to delay puberty in young animals (Wade and Haselwood, 1941), to decrease the frequency of estrous cycles in adult rats, (Corey, 1933) and to lower fertility and cause abortions (Britton and Kline, 1936). In the human female with Addison's disease, menstrual periods are often irregular or there is amenorrhea. Cases of severe adrenal deficiency in which the menstrual periods have been regular have been described however (Marañon, 1936). Complete studies of the output of sex hormones in Addison's disease have not been reported. The simultaneous presence of three women with Addison's disease in the University of Minnesota Hospitals, one of whom was pregnant at the time of admission, provided an opportunity to observe the daily fluctuation of sex hormone excretion under controlled conditions.

PROCEDURE

Patients A. G. and *E. J.* were non-pregnant women, 22 years and 37 years of age, respectively. Both had shown repeated Addisonian crises which had been relieved by administration of cortical extract. They could be maintained free of marked symptoms of insufficiency only by administration of desoxycorticosterone or by maintenance on a high sodium diet. *Case E. J.* also had a basal metabolic rate of −23 to −37 per cent. *Case E. J.* had irregular menses, but *A. G.* reported that hers had been regular. This was confirmed during 4 months of hospitalization during which time crises occurred at intervals.

Case C. W. had severe Addison's disease which had been under treatment in the outpatient department. While receiving desoxycorticosterone by implantation, she became pregnant. She was admitted to the hospital approximately 3 months after conception and remained under continuous observation until 2 months after parturition. She was maintained on a low potassium diet for a short period during early pregnancy, and then received 6 mg. of desoxycorticosterone per day until just before parturition when cortical extract was given. During pregnancy specific treatment was continued as a precautionary measure but there was little if any evidence that it was necessary. Subsequent

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to delivery Addisonian symptoms returned fully and required careful treatment. The detailed histories of these cases will be presented in another article.

Collection of urine. The urine was collected for 24-hour periods. It was kept on ice and brought to the laboratory for analysis as soon as the last specimen of the period was voided. Samples were collected daily from *A. G.* and *E. J.* for at least 30 days. Samples from *C. W.* were collected daily for 10 days and then for 2 successive days at weekly intervals until parturition, at which time the samples were collected continuously for one week. Following this another series of 48-hour samples was collected every week for a 2-month period.

Assays. Each 24-hour sample was divided into two equal portions. One-half was preserved with sodium cyanide, combined with one-half of the next day's sample, and the whole analyzed for sodium pregnanediol glucuronidate by the method of Venning (1937). The second half of each of the two specimens was combined and analyzed for estrogens and 17-ketosteroids by procedures previously described (Samuels, *et al.*, 1941).

The estrogen fraction, separated according to the methods of Gallagher, *et al.*, (1937), was then dissolved in a small amount of alcohol and dispersed in an aqueous glycol stearate emulsion according to the method of Gustavson, *et al.*, (1938). The ability to produce estrus in ovariectomized mice was then compared with that of an aqueous solution of estrone, 15 mice being used for each dosage. As mentioned in the previous paper, the values obtained by this method in mice have been found to differ greatly from assays made on rats with the same procedure. The relative estrone values are about 5 times as great in the latter animals. This appears to be because of the great sensitivity of our mice to the estrone solution, a total of .068 μg. being sufficient to produce estrus in 53 to 60 per cent of the animals. Assays are so duplicable by this method that we have continued its use.

The analyses for 17-ketosteroids were made by the method of Holtorff and Koch (1940) using the longer period of color development recommended by Nathanson (1942).

In the case of *C. W.*, most of the combined portions of urine to be assayed for estrogens and ketosteroids were further divided in half, one portion of which was hydrolyzed with acid, and extracted and assayed as previously described; in addition, the molar concentration of estrins was determined by the modification of Venning, *et al.*, (1937), of the colorimetric reaction of Kober (1931). The other portion was extracted without a preliminary acid hydrolysis and then fractionated and assayed in the same manner as the first portion. The hormones obtained by this latter procedure were considered the free hormone fraction.

By these procedures the mean total estrogenic, ketosteroid and sodium pregnanediol glucoronidate excretion for each 2-day period was obtained. With the samples from *C. W.* not only were the total estrogenic and ketosteroid values determined, but also those for the free estrogens and ketosteroids and, by difference, those for the combined compounds.

RESULTS

The results are summarized in figures 1 to 3. Figures 1 and 2 present the findings on *A. G.*, and *E. G.*, respectively, the two non-pregnant women. Both graphs show the double peaked curve of estrogen excretion characteristic of normal women (Samuels, *et al.*, 1941; Gustavson, *et al.*, 1938; Smith, *et al.*, 1936). The values while low, are within normal range. The variation in estrogen output bears no relation to the medication, since during a considerable portion of the time, therapy in the case of both *A. G.* (fig. 1) and *E. J.* (fig. 2) consisted largely of a high sodium intake. Although in the case of *E. J.*

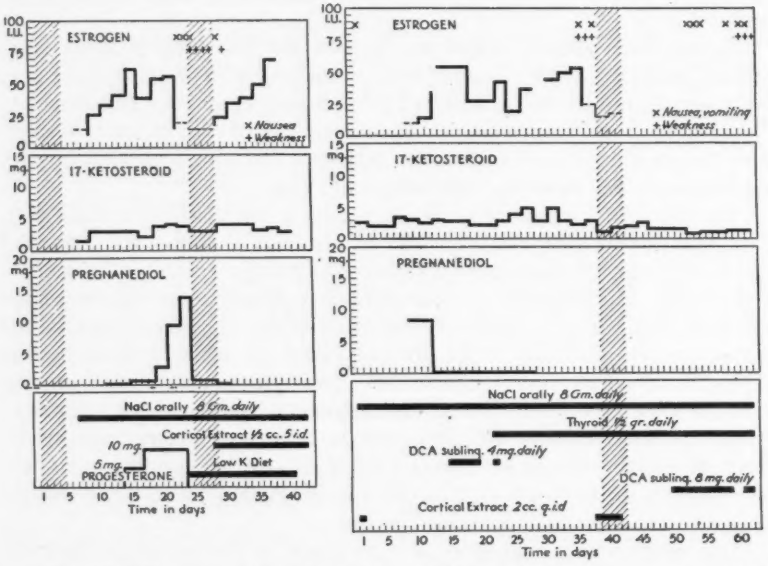

Fig. 1. (*Left*) Hormone excretion values and therapy in the case of *A. J.*, a non-pregnant woman with Addison's disease and regular menstrual cycles. The shaded area indicates the time of menstruation.

Fig. 2. (*right*) Hormone excretion values and therapy in the case of *E. J.*, a non-pregnant woman with Addison's disease and periods of amenorrhea. The shaded area indicates a menstrual period.

(fig. 2) the menstrual period indicated was the first for 3 months, one month before this the variation in estrogenic and pregnanediol output was quite similar to that seen when bleeding occurred. The only difference between conditions on the two occasions was that the patient had been given 0.25 to 0.50 grains of thyroid for a short time before menses began. This had not been sufficient to raise the basal metabolic rate to normal, determinations at this time ranging between −12 and −18 per cent.

The study of case *A. G.* began on the seventh day of a cycle and spanned one menstrual period (fig. 1). The interval between menses was fairly regular and was associated with a somewhat lengthened period of estrogenic excretion during the first phase of the estrogen cycle. This is within the normal range of variation.

The excretion of 17-ketosteroids was low in both non-pregnant women. The entire color produced in the analytical reaction could probably be accounted for by other compounds. Throughout the period of observation there was so significant variation in the ketosteroid excretion which could be attributed to therapy or to the menstrual cycle.

Unfortunately most of the sodium pregnanediol glucuronidate determinations on *E. J.* and part of those on *A. G.* were lost. The values which are available in the case of *A. G.* (fig. 1) show the presence of the compound

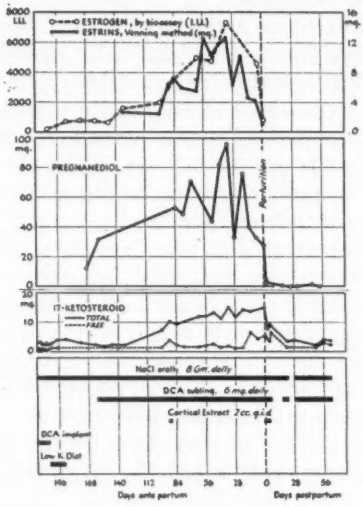

Fig. 3. Hormone excretion values and therapy in the case of C. W., a pregnant woman with Addison's disease.

during the latter half of the cycle and its disappearance at the time of menstruation; 10 mg. of progesterone had been given daily during the latter half of the cycle. A large amount of pregnanediol was excreted even though the symptoms of adrenal insufficiency were not prevented by this therapy.

Figure 3 illustrates the hormone excretion during pregnancy in the case of *C. W.* As mentioned previously, urine specimens were not collected continuously except during the first 10 days of hospitalization and at the time of parturition.

The total estrogens rose normally during pregnancy and then dropped rapidly just before the birth of the baby.

The excretion of sodium pregnanediol glucuronidate was also normal for pregnancy. The general level of 80 to 100 mg. maintained during the last two months of pregnancy is well within normal limits (Browne, *et al.*, 1938; Bachman, *et al.*, 1941). On two occasions the level fell below this range. On one of these, the levels of the ketosteroids and estrogens were also below the expected values which probably indicates that the urine collected did not

represent that for a full 48-hour period. The other variation is unexplained, since it is not correlated with changes in the other components, nor with variations in therapy. During the last 2 weeks of pregnancy the excretion of sodium pregnanediol glucuronidate began to fall. During the 48-hour period which included the day before, and the day of parturition, the excretion was 28 mg. per 24 hours, but during the next 48-hour period it was 2.5 mg. On the fifth and sixth days following parturition, an average excretion of 2.3 mg. per 24 hours was recorded, after which none was found in the urine until just before the next menstrual period.

The 17-ketosteroid excretion in this patient was distinctly abnormal. From the time when the urine was first analyzed until 3 months before parturition the ketosteroids were so low that they probably represented only color due to non-ketosteroid compounds. The color in the unhydrolyzed samples was very low. The values for the 48-hour sample collected on July 15th, 97 days before delivery, showed a marked rise over previous values, however. This increase continued during the succeeding month until by August 19 and 20 the level was 12 mg. per 24 hours, well within the range of excretion of the normal non-pregnant woman. Excretion then plateaued within a range of 11.3 to 15 mg. per 24 hours until parturition, when the output began a steady decline, reaching the original insignificant level within two weeks.

The 17-ketosteroids extractable before hydrolysis ('free' ketosteroids) represented 20 per cent of the total ketosteroids from the earliest determinations through the period of rising values. In the samples collected two weeks before parturition, however, the free ketosteroids were 47 per cent of the total. From that time until 8 days after birth, the free ketosteroids made up approximately 50 per cent of the total. They then returned to the original smaller proportion.

DISCUSSION

There is little agreement regarding the relation between the adrenals and the ovaries and placenta. A review of the subject was published by Looney (1940). Gynecomastia and feminization have been observed in men with tumors of the adrenal cortex (Lisser, 1936; Glass, 1938); women usually show masculinization as a result of the tumor. Marañon (1936) believed that the degree of irregularity in the menstrual periods of patients with Addison's disease, and the occurrence of these changes before the symptoms of the disease clearly developed, indicated that the adrenal cortex either acted with, or through, the ovary. However, in most of these experiments non-specific salt therapy was not used, and the general disturbances in cellular metabolism may have been responsible for changes in the sensitiveness of the end organs as well as failure of normal pituitary function. Nevertheless, such observations have given rise to the supposition that the adrenals might be involved in the normal function of the gonads.

Our results indicate that, in the human female, the ovaries are not under any direct control of the adrenal gland, either for basic supply or cyclic vari-

ation. The patients in this study all had such severe adrenal insufficiency that control either by regulation of salt intake or by administration of cortical hormone was essential to life. Yet there was no correlation between the administration of cortical hormone and the excretion of estrogen and pregnanediol. The excretion of these compounds was high at times when the adrenal insufficiency was controlled solely by salt therapy, while on several occasions it was low when cortical steroids were being given. The variation in excretion in the two non-pregnant women was characteristic of the normal ovarian cycle and bore no apparent relation to any external factor. Pregnanediol excretion appeared to be increased after injections of progesterone. In the pregnant woman the output of these compounds in the urine followed a normal course. Apparently the production and metabolism of estrogenic and corpus luteum hormones in women with Addison's disease is not greatly affected by the reduction in cortical steroids. Instead, our findings confirm the views expressed by others (Rowntree and Snell, 1931) that the major effect of adrenal insufficiency on ovarian function is due to the general reduction in metabolism following the disturbance in salt and water balance, and not to a specific influence as postulated by Marañon. This, like other debilitating factors, reduces the function of all organs of the body. This would seem to be the explanation for the results in *case E. J.*, in whom, without changing cortical therapy, the administration of a small amount of thyroid extract was associated with a development of the uterus and menstruation. The ovaries of this woman had been undergoing a normal cycle previously, but apparently the reduced metabolic rate of the uterus prevented the development of the endometrial conditions which are a necessary prelude to menstruation.

These findings do not rule out the possibility that the adrenal cortex is an additional source of significant amounts of these compounds since, as pointed out, the estrogen levels in the non-pregnant women were lower than the average which we have found in normal women.

As a number of workers have reported (Callow, 1938) the excretion of 17-kestosteroids was very low in the absence of adrenal cortex function. Our results confirm the findings of Fraser, *et al.*, (1941), that the administration of desoxycorticosterone apparently does not increase the output of 17-ketosteroids. In all three subjects there were periods when only salt was administered, as well as periods of desoxycorticosterone acetate therapy. There was no significant effect upon the ketosteroid excretion.

The striking feature in this series of studies is the rise in ketosteroid output beginning in the sixth month of pregnancy. This is in accord with the time at which the fetal adrenal shows some development. Since the ketosteroids dropped to their original low level following parturition, it is possible that the source of these was the adrenals of the fetus. It is significant in establishing the independence of the ovary and placenta to note that there is no relation between the ketosteroid excretion, which is related to adrenal function, and the excretion of estrogen and sodium pregnanediol glucuronidate.

SUMMARY

In 2 non-pregnant women suffering from Addison's disease the estrogen excretion, while low, showed the normal double peak; sodium pregnanediol glucuronidate appeared during the latter half of the cycle. Since the amount of functioning adrenal cortex in severe Addison's disease is very small, and since in these cases the 17-ketosteroid excretion indicated that cortical function was very low, it is concluded that ovarian function cannot to any major degree be dependent upon a correlated rhythm in the adrenal cortex. As long as electrolyte balance is maintained the pituitary may, therefore, carry on the normal periodic production of gonadotropic hormones in the absence of sufficient adrenal cortex hormone to maintain life without salt therapy.

In one case of Addison's disease studied during pregnancy, the excretion of estrogen and sodium pregnanediol glucuronidate showed the smooth progressive increase characteristic of normal pregnancy. The excretion of these compounds began to drop just before parturition and returned to normal non-pregnant levels thereafter.

The excretion of 17-ketosteroids in the pregnant woman remained very low until the sixth month of pregnancy and then rose to a level of 11 to 15 mg. per day. It immediately fell to the original low level after birth of the child. Just before parturition the proportion of 'free' 17-ketosteroids increased from a level of approximately 20 per cent to 50 per cent of the total but returned to the original 20 per cent within a week after childbirth.

It is concluded from these studies that the ovary and placenta undergo their normal rhythm and can produce their normal secretory products in severe Addison's disease as long as the individual is maintained in good general metabolic condition by appropriate therapy.

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STUDIES ON THE FIXATION OF RADIOACTIVE IODINE BY THE RABBIT THYROID[1]

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THE GREAT RAPIDITY with which the thyroid concentrates iodine following the administration of soluble iodides would seem to involve a consideration of chemical transformations occurring within this gland. Recent data (Perlman, *et al.*, 1941; Morton, *et al.*, 1941; Mann, *et al.*, 1942; Morton, *et al.*, 1942), obtained with radioactive iodine, have shown that circulating iodide is rapidly incorporated into the diiodotyrosine and thyroxin fractions of thyroid protein. In the shortest interval employed, Mann, Leblond, and Warren (1942) found considerable quantities of labeled iodine in the organic fractions (particularly the diiodotyrosine fraction) as early as 30 minutes following the administration of tracer doses of radioactive iodide.

In studying the rate of fixation of administered (exogenous) iodide a number of investigators (Marine and Feiss, 1915; Hertz, *et al.*, 1940; Hamilton and Soley, 1940; Lein, 1941; Leblond and Sue, 1941) have determined that the rate of concentration of iodine by the thyroid is greatest during the first 10 minutes following administration. A study of the reactions occurring in the thyroid gland immediately following the administration of iodides might be expected to throw some light on the nature of the thyroid's iodine-fixing mechanism. In the present investigation, therefore, observations have been made on the accumulation of labeled iodine in the inorganic and organic fractions of the thyroid gland with particular emphasis on the accumulation which occurs during the first 60 minutes following the administration of radioactive iodide.

PROCEDURE

Radioactive iodine,[2] predominantly the 8-day isotope (I^{131}), was produced by deuteron bombardment of tellurium in the cyclotron. The tellurium target was dissolved in concentrated HNO_3, from which the radioactive iodine was distilled into CCl_4. The distillate was washed with dilute $Na_2S_2O_3$ thereby bringing the radioactive iodine into aqueous solution as an iodide. Since these studies were to be made on administered (exogenous) iodide, tracer doses of radioactive iodide were not employed. Stable iodide was added to the radioactive material to give about 35 micrograms for each dose used. This quantity was chosen because it was estimated to yield an increase in the blood iodide

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of the test rabbits within the range which might be expected following the ingestion of a meal containing an adequate iodine content.

Each dose of 35 μg. of stable iodide labeled with radioactive iodide was injected intravenously into normal healthy mature male rabbits which had been kept in the laboratory for at least 10 days and which had not been fed for 36 hours prior to use. At various time intervals after injection, the animals were killed, the thyroid glands rapidly dissected out, weighed and placed in small volumes of acetone. After dehydration by acetone, each thyroid was minced and both acetone and thyroid were placed in a micro-Soxhlet extractor in which extraction was permitted to continue for 5 hours. Both the soluble fraction and insoluble fraction were analyzed for radioactivity and for total iodine. Preliminary test fractionations with the acetone procedure employed here indicated that inorganic iodide is quantitatively removed by the acetone while thyroglobulin iodine remains in the acetone-insoluble fraction.

Determination of radioactivity was made with a Geiger-Müller counter after digesting the samples with chromic acid and sulfuric acid according to the procedure of Matthews, Curtis and Brode (1938) and distilling the iodine in a Chaney (1940) still. Rigidly standardized procedure in using the Geiger-Müller counter permitted the interpretation of radioactivity in terms of micrograms of injected iodide.

RESULTS

In the group of 10 rabbits employed in this study the size of the thyroid and its iodine concentration varied considerably. The thyroid weights ranged from 149 to 550 with an average of 317 mg. The iodine concentration varied from 8.7 to 131.6 and averaged 76.3 mg. per cent. The accumulation of labeled iodine by the thyroid seemed to be independent of the total iodine concentration of that gland, but appeared to be dependent on both the size of the gland and length of time permitted for accumulation to occur. The results, therefore, are expressed in terms of concentration of iodine for each time interval investigated.

As has been repeatedly demonstrated, the thyroid was found to accumulate more iodine per unit weight than any other tissue. The appearance of labeled iodine in tissues other than the thyroid was in accord with the data of Ariel, *et al.*, (1941). The accumulation of labeled iodine by the lungs of the test animals was particularly noteworthy in that it was constantly high and second only to that in the thyroid. One experiment in which exhaled air was trapped and measured for radioactivity indicated that very little iodine is excreted by way of the lungs.

During the first few minutes following the intravenous administration of the labeled iodide, the rate of concentration by the thyroid gland is rapid. As indicated in figure 1, this initial rapid accumulation of iodine occurs in the acetone-soluble (inorganic) fraction and reaches its highest concentration at the end of about 10 minutes. Thereafter, the concentration of labeled iodine in this fraction slowly decreases.

The conversion of radioactive iodine from the inorganic to the organic form is rapid since radioactive iodine is found in the acetone-insoluble (organic) fraction as early as 5 minutes following intravenous injection of the labeled iodide. The curve indicating appearance of organically bound radioactive iodine rises rapidly so that at the 30-minute interval, half of the thyroid's total accumulation is in the organically bound form. Although labeled iodine accumulates rapidly in this fraction for several hours, the rate of accumulation slowly decreases.

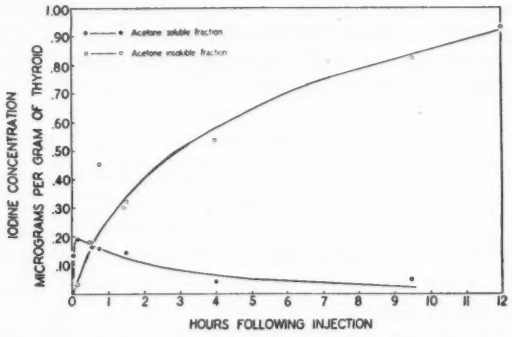

Fig. 1. Concentration of acetone-soluble and acetone-insoluble labeled iodine in the rabbit thyroid after the intravenous administration of 35 mg. of soluble labeled iodide.

DISCUSSION AND CONCLUSIONS

Immediately following the intravenous administration of labeled iodide, the acetone-soluble (inorganic) iodine of the thyroid increases rapidly to a level higher than that of other tissues. Since the highest concentration of this fraction is reached in approximately 10 minutes, this would indicate that the iodide-combining mechanism of the thyroid is saturated within that time. This is in accord with the work of previous investigators (Marine and Feiss, 1915; Hertz, *et al.*, 1940; Hamilton and Soley, 1940; Lein, 1941). It should be mentioned that this saturation point holds only for the dose of iodide injected, for as Hertz, *et al.*, (1938) and Leblond and Sue (1941) have shown, the quantity of iodide fixed by the thyroid is dependent on the quantity administered.

The data presented in figure 1 seem to indicate that the mechanism for this initial rapid fixation of labeled iodide is not dependent on the conversion of such iodide into a protein-linked form. Protein-linked iodine has been shown in our laboratory to be acetone-insoluble whereas most of the iodine fixed during the first few minutes following iodide administration has been found to be acetone-soluble (fig. 1). The mechanism, therefore, for this initial rapid fixation of iodide would appear to involve a loose bond with iodide so that its solubility in acetone is not lost.

The initial rapid fixation of iodide of itself will not account for the large

quantities of iodine found within the thyroid. An examination of figure 1 shows that at the end of 12 hours the thyroid contains between 4 and 5 times as much iodine as was initially fixed by the gland. The conversion of the inorganic iodine to an organically linked form appears to be responsible for this continued accumulation. The conversion begins immediately following the administration of iodides, for small quantities of acetone-insoluble labeled iodine were found as early as 5 minutes following the administration of labeled iodide. It appears likely that as the thyroid releases acetone-soluble (inorganic) iodine to the acetone-insoluble (organically linked) form, its capacity to fix circulating iodide is slowly renewed. This is evidenced by the fact that iodine appears in the acetone-soluble fraction for several hours. The slow decrease in this fraction following its initial rapid rise may reflect the decreasing concentration of circulating blood iodide.

SUMMARY

The thyroid glands of rabbits were fractionated at various time intervals following the intravenous administration of labeled iodide. The results in the first 60 minutes were emphasized because the thyroid fixes iodine most rapidly immediately after administration. The most rapid accumulation of iodine occurs during the first 10 minutes following the injection and the iodine fixed is acetone-soluble.

Acetone-insoluble labeled iodine is found in small quantities as early as 5 minutes following the injection, and the quantity rapidly increases. The total quantity of iodine in the acetone-insoluble fraction at the end of 12 hours is 4 or 5 times greater than that initially fixed by the thyroid.

It appears likely that as fixed iodide is converted into a protein-linked form, the previously saturated 'fixing mechanism' may renew its capacity to mobilize circulating iodide.

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INFLUENCE OF HYPERTHYROIDISM ON URINARY EXCRETION OF THIAMIN AND RIBOFLAVIN[1]

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IN PREVIOUS publications it was demonstrated that vitamin B complex concentrates (Sure and Smith, 1934) and crystalline thiamin (Sure and Buchanan, 1937) offer protection against loss of body weight of the albino rat in hyperthyroidism which is produced by subcutaneous injections of 0.05 to 0.25 mg. of natural thyroxin; also that hyperthyroidism produces considerable loss of ascorbic acid in various tissues (Sure and Theis, 1939). In this and in previous studies the existence of hyperthyroidism was determined by loss of body weight, cardiac hypertrophy and marked creatinuria

In the present communication experimental data are submitted on the influence of subcutaneous administration of synthetic thyroxin on urinary excretion of thiamin and riboflavin. Results of this investigation are presented in tables 1 and 2.

EXPERIMENTAL PROCEDURE

Four pairs of litter mate rats, weighing 170 to 185 gm. and 64 days old, were placed on a purified ration of the following composition: casein (vitamin-free, Smaco), 18; agar-agar, 2; Sure's salts No. 1 (1941), 4; butter fat, 10; and dextrin, 66. This diet was supplemented with a daily allowance of 20 μg. of thiamin, 20 μg. of riboflavin, 20 μg. of pyridoxin, 6 mg. of choline chloride, and 200 μg. of calcium pantothenate. After 3 weeks on this ration, the animals had gained 40 to 80 gm. each. Following the preliminary feeding period on the purified ration, one animal in each pair was injected daily with 0.5 mg. of synthetic thyroxin for 14 days, after which the dose was increased to 1.0 mg. daily for another 3 days. The experiment was discontinued on the 18th day when two of these animals died. The average daily dose of thyroxin was 0.59 mg.

This study was conducted with increasing amounts of thiamin. The first pair received 20 μg. daily of this vitamin, the second pair, 50 μg., and the third and fourth pairs 100 μg. The fourth pair also received 20 mg. daily of ascorbic acid, because of the claim for Fischer and Oehme (1937) that the administration of large amounts of vitamin C to rats suppressed the creatinuria induced

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by thyrotoxicosis. The daily doses of riboflavin and pyridoxin were increased to 50 μg.

Urinary excretion of thiamin and riboflavin was also studied in another group of 6 pairs of male animals, 170 to 190 gm. in weight, and 68 days old, which were injected subcutaneously for 21 days. However, the results obtained with this group are very similar to those secured with the first group (table 1) so that detailed data are not presented. The technique used for the

TABLE 1. INFLUENCE OF HYPERTHYROIDISM ON URINARY EXCRETION OF THIAMIN AND RIBOFLAVIN IN MALE RATS

ANIMAL	CHANGE IN BODY WEIGHT	TOTAL FOOD INTAKE	THIAMIN				RIBOFLAVIN		
			DAILY THIAMIN INTAKE	TOTAL THIAMIN INTAKE	THIAMIN EXCRETED	THIAMIN EXCRETED	TOTAL RIBOFLAVIN INTAKE	RIBOFLAVIN EXCRETED	RIBOFLAVIN EXCRETED
	gm.	gm.	μg.	μg.	μg.	%	μg.	μg.	%
1C	+26	211.6	20	340	14.44	4.2	850	83.09	9.7
2E	−42	220.4	20	340	18.65	5.2	850	220.96	26.0
3C	+21	211.5	50	850	17.96	2.1	850	76.41	9.0
4E	−17	208.6	50	850	20.00	2.4	850	200.47	23.6
5C	+14	200.6	100	1,700	40.80	2.4	850	96.96	11.4
6E	−45	232.8	100	1,700	47.64	2.8	850	313.76	36.9
7C[1]	+14	192.4	100	1,700	54.90	3.2	850	198.82	23.4
8E[1]	−59	244.2	100	1,700	56.93	3.3	850	410.61	48.2

[1] Supplemented with 20 mg. of ascorbic acid daily.

Experimental period, 17 days; average daily dose of synthetic thyroxin, 0.59 mg.
C = control; E = experimental.

determination of thiamin and riboflavin in urine has been described in a recent communication (Sure and Ford, 1942).

RESULTS

It is apparent from table 1 that during the 17 days of thyroxin injections there was only a slight loss of thiamin but a large loss of riboflavin in the urine of the treated animals. The animals receiving thyroxin excreted approximately 3 times as much riboflavin as the littermate controls that did not receive thyroxin. It is also evident that administration of 20 mg. of ascorbic acid daily accentuated the large amount of urinary riboflavin caused by the thyrotoxicosis. From the loss of body weight of the animal that received thyroxin, it would appear that the high level of riboflavin excreted in the urine was associated with body tissue catabolism. Hence the second group of 6 pairs of male rats, on which urinary studies were made of thiamin and riboflavin excretion, were killed following a 23-day experimental period during which one animal in each pair received 1.0 mg. of synthetic thyroxin daily. The various tissues of the experimental animals were pooled, as were those of the control rats and these were analyzed for thiamin and riboflavin content by the method of Conner and Straub (1941).

From table 2 it is evident that hyperthyroidism produced a much greater decrease in riboflavin than thiamin in the various tissues of the treated rats. The greatest decrease of riboflavin was in the muscle, heart, brain, lungs, testes and the gastro-intestinal tract. Since hyperthyroidism produced negligible loss of thiamin in the urine, how could a decrease of this vitamin be accounted for in the liver, spleen and small intestine? It was found that incubation of liver, lung, heart, stomach, small intestine, and pancreas at 40° C.

TABLE 2. INFLUENCE OF HYPERTHYROIDISM ON THIAMIN AND RIBOFLAVIN CONTENT OF TISSUES. AVERAGE RESULTS ON 6 PAIRS OF ADULT MALE ALBINO RATS

TISSUES	THIAMIN CONTENT OF TISSUE			RIBOFLAVIN CONTENT OF TISSUE		
	EXPERIMENTAL	CONTROL	PERCENTAGE CHANGE IN EXPERIMENTAL	EXPERIMENTAL	CONTROL	PERCENTAGE CHANGE IN EXPERIMENTAL
	μg./gm.	μg./gm.		μg./gm.	μg./gm.	
Liver	4.09	6.00	−31.8	38.5	43.4	−11.0
Kidney	12.32	12.02	+ 2.4	126.5	145.4	−13.0
Spleen	1.62	2.14	−24.3	14.6	14.7	− 0.7
Heart	6.73	7.39	− 8.9	28.2	45.1	−37.5
Lung	3.95	4.09	− 3.4	11.0	16.4	−32.9
Brain	7.15	7.67	− 6.8	9.6	13.7	−29.8
Testes	15.08	17.93	−15.9	15.6	30.7	−49.2
Stomach	2.26	2.09	+ 8.1	10.5	22.6	−53.5
Small intestine	1.69	2.26	−25.2	13.2	17.5	−24.6
Large intestine	2.03	1.72	+12.2	9.5	16.9	−43.8
Pancreas	0.73	0.72	+ 1.3	21.1	19.6	+ 7.8
Muscle	0.49	0.51	− 3.9	1.17	3.72	−68.5

1.0 mg. synthetic thyroxin for 21 days.

for 48 hours resulted in a destruction of thiamin to the extent of 14, 17, 12, 26 and 20 per cent, respectively. The decrease of thiamin content in several of the tissues may, therefore, have been caused by destruction by the tissues.

Although subcutaneous injections of 1.0 mg. daily of synthetic thyroxin resulted in a decrease of the riboflavin content of most of the tissues, oral administration of the same amount of synthetic thyroxin daily to 6 pairs of rats, and 1.0 mg. daily of natural thyroxin[2] to another 6 pairs of animals, produced only a small decrease of either thiamin or riboflavin from the various tissues. That the latter animals receiving thyroxin developed hyperthyroidism was evident from marked creatinuria and cardiac hypertrophy, but the loss of body weight was small. Such differences in results among the various groups of animals may be due to the greater absorption of thyroxin parenterally than orally since the animals that were injected with thyroxin apparently received greater damage than those that received thyroxin by mouth and hence these lost more riboflavin from their tissues.

SUMMARY

Subcutaneous injections of 0.5 to 1.0 mg. of synthetic thyroxin to adult albino rats for 17 to 21 days resulted in the excretion of large amounts of

[2] The thyroxin was kindly furnished by Dr. R. J. Anderson, Director of Biological Laboratories, E. R. Squibb & Sons, New Brunswick, N. J.

riboflavin in the urine. Since hyperthyroidism also produced large losses of body weight and of riboflavin from many of the tissues, the increased excretion of riboflavin in the urine may have been caused by body tissue catabolism. Hyperthyroidism, however, caused negligible changes in the level of thiamin in the urine and much smaller losses of this vitamin from the tissues.

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SODIUM RETENTION IN THE OPOSSUM[1]

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IN A MAJORITY of the mammals which have been studied the adrenal maintains the plasma sodium at a higher level than that found after adrenalectomy. Whether the animal is adrenalectomized or not, the injection of adrenal extracts or the synthetic compound, desoxycorticosterone acetate, causes retention of sodium. From the work of Silvette and Britton (1936) and the more recent investigation in our own laboratory (Hartman, *et al.* 1943) it appears that this does not occur in the opossum. Following adrenalectomy there is little change in plasma electrolytes, even in animals surviving for many months, except during such a stress as diarrhea or lactation (Britton and Silvette, 1937).

If the opossum adrenal produces a sodium factor the animal should respond to injections of the same. In this paper the results are reported of a study of the influence of adrenal preparations and of desoxycorticosterone on sodium in the plasma.

METHODS

Twenty-five female opossums (*Didelphys virginiana*) were used in this investigation, all being furnished by the Wistar Institute. Eleven were wild animals, while 14 were raised by the Institute. The latter were 1 to 3 years old when used for experiment, having been in our laboratory since they were about 6 months of age. The animals were kept in individual cages, and were fed 150 gm. daily of the diet recommended by the Wistar Institute (Hartman, *et al.*, 1943). Blood was obtained by heart puncture, heparin being used as an anticoagulant. The methods of analysis have been described previously (Hartman, *et al.*, 1943).

Unfractionated adrenal extract was prepared from whole beef glands by the acetone method (Cartland and Kuizenga, 1936). It contained the sodium factor as well as other active substances. Each cc. caused a sodium retention equivalent to that produced by 1 mg. of desoxycorticosterone acetate in a dog. The amount of sodium factor fraction (Hartman and Spoor, 1940) injected daily produced a sodium retention in the dog equivalent to that for 1 mg. of desoxycorticosterone acetate.

In the studies of electrolyte balance each animal received 75 gm. of the special diet so that all would be consumed. This was sufficient to maintain body weight. There was free access to water. Each animal was confined in a

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metabolism cage in order to collect the urine. Catheterization was employed at the beginning and at the end of a 24-hour period.

RESULTS

Response to unfractionated adrenal extract. Unfractionated adrenal extract was injected subcutaneously for 3 days. At 8 A.M. 3 cc. were given; at

TABLE 1. INFLUENCE OF ADRENAL PREPARATIONS ON BLOOD AND URINE OF NORMAL OPOSSUMS

ANIMAL	TREATMENT[1]	BLOOD PLASMA[2]			ERYTHROCYTES	URINE FOR 72 HOURS			
		SODIUM	POTASSIUM	CHLORIDE		VOL.	SODIUM	POTASSIUM	CHLORIDE
					vol. %	cc.	m.eq.	m.eq.	m.eq.
1, 2.2 kg.	Control values	139.9	5.5	121.0	47	165	18.2	29.0	28.8
	Unfractionated extract 7.5 cc.	139.9		88.0	45	110	11.0	19.3	16.0
	Na factor	139.7	5.6		48	165	22.1	23.3	29.1
	DCA 1 mg.	154.5	3.5	103.0	44	118	14.6	31.2	19.6
7, 2.6 kg.	Control values	144.3	4.2	106.0	48	235	31.6	21.4	37.0
	Unfractionated extract 7.5 cc.	144.2	4.2	97.0	39	195	25.5	19.9	31.3
	Na factor	142.3	6.5		44	200	25.4	24.8	31.2
	DCA 1 mg.	152.5	5.5	104.2	46	225	23.8	25.5	30.0
3, 2.5 kg.	Control values	143.0	4.8	101.0	50	207	18.5	27.2	26.9
	Unfractionated extract 7.5 cc.	149.8	5.5	117.0	50	335	36.8	33.5	40.5
	Na factor	142.3	5.2		51	350	23.8	23.3	31.6
	DCA 1 mg.	153.8	6.0	105.0	48	103	14.3	24.4	14.9
5, 2.4 kg.	Control values	139.9	5.1	103.4	48	280	19.1	23.6	29.1
	Unfractionated extract 7.5 cc.	138.3	4.2	93.0	47	195	26.5	27.8	29.5
	Na factor	143.3	4.2		47	238	39.5	28.8	38.7
	DCA 1 mg.	152.2	5.0	107.0	38	230	24.4	30.0	23.8
10, 2.4 kg.	Control values	139.9	5.6	115.0	45	166	25.2	28.5	29.6
	Unfractionated extract 7.5 cc.	142.2	5.9	107.0	44	260	29.2	28.5	35.3
	Na factor	138.6	5.2		43	210	20.0	28.8	29.6
	DCA 1 mg.	143.5	4.4	99.8	42	233	21.8	34.6	32.3
12, 2.3 kg.	Control values	141.2	4.8	101.0	45	190	23.7	23.9	29.3
	Unfractionated extract 7.5 cc.	140.5	4.7	101.0	42	230	33.2	20.6	36.7
	Na factor	143.5	5.0		46	245	28.1	18.8	33.1
	DCA 1 mg.	140.1	5.3	97.0	43	260	25.6	33.5	31.2

[1] Preparations injected daily for 3 days.
[2] Electrolyte values expressed in m.eq./l.

5 P.M., 2 cc.; and at 11 P.M., 2.5 cc. The potency was such that the sodium retention produced by the same amount of extract in the dog was equivalent to that for 7.5 mg. of desoxycorticosterone acetate. The total quantity of urine for the whole period and a blood sample at the end of the period were analyzed. These values were compared with those obtained in control periods of the same length just before the experiment. Eight Wistar opossums were studied and the results from all except *no.* 2 and 6 are shown in table 1.

At the end of the 3-day period, there were no significant changes in plasma sodium and potassium concentrations with the exception of 1 (*no.* 3), which showed an increase in sodium. Plasma chloride concentrations were unchanged in 2 (*no.* 6 and *12*), increased in 1 (*no.* 3) and decreased in the other 5. Assuming that the total volume of erythrocytes remained unchanged, there appears to have been an actual increase of total plasma sodium in 5 of the 8 animals (*no.* 1, 2, 7, *10* and *12*).

For the 3-day period the volume of water excreted was reduced in 5 animals (*no.* 1, 2, 5, 6 and 7) and increased in 3, (*no.* 3, *10* and *12*). All urinary electrolytes were reduced in 3 (*no.* 1, 6 and 7) and increased in 1 (*no.* 3). Urinary sodium alone was increased in the other 4 (*no.* 2, 5, *10* and *12*). Potassium was decreased in 4 (*no.* 1, 2, 7 and *12*), increased in 2 (*no.* 3 and 5) and little changed in 2 (*no.* 6 and *10*). Chlorides were decreased in 2 (*no.* 1 and 7), increased in 4 (*no.* 2, 3, *10* and *12*) and unchanged in 2 (*no.* 5 and 6). These observations indicate that although adrenal extract may produce moderate sodium retention in some individual animals, it is small compared with the effect of the same amount of extract in dogs.

Sodium factor fraction. This fraction, in oil, was injected daily for 3 days into the same opossums tested with unfractionated extract. Blood samples and urine collections were made for the same periods and comparisons with similar control periods were made.

At the end of the 3-day period (table 1), there was little effect on the concentration of sodium in the plasma except a moderate increase in *no.* 5 and greater increase in *no.* 6 (149.6 m.eq./l. from 140.3). Changes in potassium concentrations were not remarkable for the most part. The volume per cent of erythrocytes was reduced significantly in 3 animals (*no.* 2, 7 and *10*).

For the 3-day period the volume of water excreted was decreased in 3 animals (*no.* 2, 5 and 7); increased in 3 (*no.* 3, *10* and *12*) and remained unchanged in 2 (*no.* 1 and 6). The excretion of sodium was decreased in 4 (*no* 2, 6, 7 and 10); and increased in 4 (*no.* 1, 3, 5 and *12*). The excretion of potassium was decreased in 5 (*no.* 1, 2, 3, 6 and *12*); increased in 2 (*no.* 5 and 7) and unchanged in 1 (*no.* *10*). The excretion of chlorides was decreased in 3 (*no.* 2, 6 and 7); increased in 3 (*no.* 3, 5 and *12*) and unchanged in 2 (*no.* 1 and *10*)

The effects of this fraction were so variable that one must conclude that sodium factor is not very potent in the opossum. It will be recalled that the amount injected was equivalent to 1 mg. of desoxycorticosterone acetate.

Desoxycorticosterone acetate. Injections of 1 mg. daily of desoxycorticosterone into the same 8 opossums tested with unfractionated adrenal extract and with the sodium factor fraction produced the following effects.

At the end of the 3-day period the plasma sodium concentration rose in 6 animals (*no.* 1, 3, 5, 6, 7 and *10*); fell from 139 to 130.5 m.eq./l. in 1 (*no.* 2), and remained unchanged in another (*no.* *12*). The changes in potassium concentration were not very significant except in *no.* 1 in which it fell to 3.5 m.eq./l. The chloride concentration was quite variable. The volume per cent of erythrocytes fell in all animals, although not markedly except in *no.* 5. Typical results are given in table 1.

In the urine collected over the 3-day period, the volume was reduced in 4 (*no. 1, 3, 5* and *7*); increased in 3 (*no. 2, 10* and *12*) and remained unchanged in 1 (*no. 6*). The total sodium in the urine was reduced in 5 (*no. 1, 3, 6, 7* and *10*) and increased in 3 (*no. 2, 5* and *12*). The total potassium in the urine was increased in all but one (*no. 3*) which showed a decrease. The amount of total chlorides in the urine was decreased in 5 (*no. 2, 3, 5, 6* and *7*) and increased in 3 (*no. 2, 10* and *12*). Therefore, this dosage of desoxycorticosterone caused retention of sodium and chloride and excretion of potassium in more than half of the experiments. Larger doses were tried in order to see whether the effect could be made more general.

TABLE 2. INFLUENCE OF DESOXYCORTICOSTERONE ACETATE ON BLOOD AND URINE

ANIMAL	DOSAGE	BLOOD PLASMA[1]			ERYTHROCYTES	URINE			
		SODIUM	POTASSIUM	CHLORIDE		VOL.	SODIUM	POTASSIUM	CHLORIDES
					vol. %	*cc.*	*m.eq.*	*m.eq.*	*m.eq.*
21	Control values	142.0	8.2	108.0	50	260	30.9	31.0	30.6
	5 mg., 3 days	153.0	5.4	100.0	43	475	21.2	30.8	28.6
24	Control values	142.0	6.6	101.0		350	25.4	20.2	25.3
	5 mg., 3 days	138.0	5.1	105.0		385	27.5	35.2	30.9
33	Control values	156.0	6.9	103.0	54	571	43.1	34.7	48.0
	5 mg., 3 days	142.0	5.3	104.0	41	275	23.0	26.8	30.8
15	Control values	139.8	5.9	104.0	43	352	26.4	22.6	28.2
	3 mg., 3 days					360	50.2	23.6	25.3
	3 mg., 6 days	117.6	4.0	112.0	34	444	36.9	29.2	46.6
18	Control values	144.3	6.3	106.0	52	313	25.2	21.9	27.9
	3 mg., 3 days					255	28.7	18.4	20.0
	3 mg. 6 days	173.0	4.6	86.5	49	460	40.9	35.8	44.2
31	Control values	144.9	5.6	97.0	60	297	24.9	22.4	29.1
	3 mg., 3 days					280	32.1	24.6	21.8
	3 mg., 6 days	149.6	4.6	102.3	56	460	29.5	35.5	41.2

[1] Electrolyte values expressed in m. eq./l.

Two mg. of desoxycorticosterone acetate daily for 6 days in 2 animals, and 3 days in a third, produced no effects on urinary excretion, with the exception of one of the 2 animals receiving 2 mg. daily for 6 days; excretion was increased in this animal during the second 3-day period. The urine volume was 300 cc. (control, 220 cc.); sodium was 36 m.eq. (control, 24 m.eq.), potassium was 27.7 m.eq. (control, 18 m.eq.), chloride was 40 m.eq. (control, 25 m.eq.)

In the same animal the concentration of sodium and potassium in the plasma was reduced from 154.7 and 4.5 to 150.2 and 2.0 m.eq., respectively. This was accounted for in part by an increase in plasma volume (total volume of erythrocytes 41 from 47%). In the animal treated for 3 days with 2 mg. daily the plasma sodium rose to 150.5 from 128.3 m.eq./l. and the potassium fell to 3.6 from 5.2 m.eq./l.

Three mg. of desoxycorticosterone acetate daily for 6 days in 3 animals (table 2) produced an increase in plasma sodium concentration in 2 and a decrease in plasma sodium in 1. Plasma potassium was reduced in all. Urinary excretion of electrolytes was increased in all.

Two animals were injected with 4 mg. of desoxycorticosterone acetate daily for 6 days. One (*no.* 13) showed an increase of plasma sodium concentration to 177.8 from 144.3 m.eq./l. with a fall in volume per cent of erythrocytes to 48 from 51. All of the electrolytes in the urine were increased: sodium, 40.3; potassium, 36.9 and chloride, 42.0 from sodium 29.1; potassium, 26.0 and chloride, 30.6, m.eq. The other animal showed a reduction of plasma sodium from 145.6 to 109.6 m.eq./l. with a reduction of erythrocyte volumes per cent from 53 to 50. All of the electrolytes in the urine were increased: sodium, 45.5; potassium, 41.5 and chloride, 49.6 from sodium, 21.9; potassium, 18.9 and chloride, 20.7 m.eq.

TABLE 3. EFFECT OF A SINGLE DOSE OF DESOXYCORTICOSTERONE ACETATE (5 MG.) ON URINE EXCRETION (24-HOUR SAMPLE)

ANIMAL		VOLUME	SODIUM	POTASSIUM	CHLORIDE
		cc.	*m.eq.*	*m.eq.*	*m.eq.*
2	Control	168	16.6	12.7	19.1
	Experimental	124	8.8	8.5	11.1
24	Control	184	12.1	8.0	13.4
	Experimental	81	3.8	4.1	4.8
26	Control	186	8.5	6.7	9.9
	Experimental	74	4.6	4.4	7.1
27	Control	60	8.9	5.1	8.1
	Experimental	43	1.7	4.6	3.8
32	Control	130	9.9	7.6	11.5
	Experimental	62	3.5	6.5	4.6

The effect of a single dose of 5 mg. of desoxycorticosterone acetate was studied in 5 animals (table 3). The electrolytes in the urine were reduced in all to a marked degree. The volume of urine was also reduced.

The same dose given daily for 3 days in 3 animals caused variable results in the urine excretion. There was sometimes a decrease, sometimes an increase, in each electrolyte. Likewise, the blood picture was somewhat variable. The plasma sodium was increased in 1 and decreased in 2. The plasma potassium was decreased in all 3 (table 2).

These observations would indicate that the first effect of desoxycorticosterone is retention of sodium followed later by increased excretion. On the other hand potassium excretion is facilitated from the first.

DISCUSSION

Silvette and Britton (1938) found that adrenal extract caused retention of sodium in adrenalectomized opossums given 0.1 per cent NaCl *per os*.

In our experiments the sodium factor, either in unfractionated adrenal

extract or as a precipitated product, produced only moderate sodium retention in some animals and none in others. Desoxycorticosterone acetate caused sodium and potassium retention at first, to be followed later by their excretion. The failure of the normal opossum to maintain a consistent retention with adrenal extract or with the sodium factor fraction would indicate that the structures affected by this factor are relatively insensitive. The amounts of adrenal extract or sodium factor employed were sufficient to produce marked sodium retention in a normal dog. Desoxycorticosterone did cause a retention of plasma sodium during the first 24 hours but this was not specific since potassium was likewise retained. Later the usual diuretic effect might occur with an increase in sodium, water, potassium and chloride excretion.

Attention should be drawn to the fact that the unfractionated extract was less potent than desoxycorticosterone.

From these observations and from the fact that there is no significant change in electrolyte metabolism after adrenalectomy, it seems that sodium retention is a much less important adrenal function in the opossum than it is in other mammals.

SUMMARY

The effects of adrenal extract, sodium factor fraction and desoxycorticosterone were studied in the normal opossum. Adrenal extract produced moderate sodium retention in some animals but this was considerably less than would be produced by the same amount of extract in dogs. The precipitated sodium factor likewise possessed little potency. Desoxycorticosterone acetate was more effective, causing retention of sodium at first, to be followed later by excretion. The opossum appears to be less responsive to these sodium-retaining substances than are other mammals.

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EFFECT OF THE INJECTION OF SEX HORMONES ON SERUM AND MUSCLE ELECTROLYTE OF RATS

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THE INVESTIGATIONS of Thorn and Engel established the fact that the sex hormones are similar to desoxycorticosterone acetate and to some of the other hormones of the adrenal cortex, in that single injections are followed by a decreased renal excretion of sodium and chloride and an increased renal excretion of potassium (Thorn, *et al.*, 1938 a, b). None of the sex hormones was found by these investigators to be as potent as desoxycorticosterone acetate in its effect on the renal excretion of sodium, potassium and chloride; considerable quantitative differences were noted among the sex hormones themselves in this respect. Kenyon and his collaborators demonstrated that the repeated injections of testosterone propionate in castrated men and normal men and women were followed by a retention of sodium, chloride, potassium, inorganic phosphorus, total urinary nitrogen and water and that there was an increase in body weight (Kenyon, *et al.*, 1938, 1940). Similar electrolyte changes were found by Thorn and Engel (1938 b) in normal dogs and in one male human subject following repeated injections of large doses of the same hormones. Since some of these effects on electrolyte metabolism were absent following the injection of desoxycorticosterone acetate or progesterone, it was apparent that differences as well as similarities existed between the two groups of hormones and among the sex hormones themselves. A further difference was claimed by Thorn and Engel because single injections of α-estradiol were followed by a decreased renal excretion of inorganic phosphorus and total nitrogen and yet α-estradiol had a greater effect on potassium excretion than testosterone propionate. From the foregoing review it is seen that the differences among the hormones in question are almost as pronounced as their similarities. Since it has been clearly shown that desoxycorticosterone acetate in normal animals brings about the replacement of intracellular potassium of the skeletal muscle with sodium (Miller and Darrow, 1941; Ferrebee, *et al.*, 1941; Darrow and Miller, 1942), it was felt that a study of the effect of the sex hormones on this particular aspect of electrolyte metabolism would give additional information concerning their actions on electrolyte metabolism in general.

Increases in renal weight and total solids have been observed following the injection of testosterone propionate and desoxycorticosterone acetate

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TABLE 1. EFFECT OF SEX HORMONES ON SERUM ELECTROLYTE AND KIDNEY WEIGHTS

TREATMENT			AVERAGE WEIGHT CHANGE %	SERUM ELECTROLYTE[1]			
DAYS	TOTAL AMT. MG.	NO. OF RATS		PER L ULTRAFILTRATE		PER L SERUM	H_2O %
				Na	Cl	K	
Group I, males, controls							
Control		12		144.9 ±3.13[2]	110.8 ±4.12	4.0 ±.73	94.04 ±.37
Group II, males, testosterone propionate							
14	14	2	+2	145.5	105.7	6.4	94.55
26	28	2					
Group III, males, estradiol dipropionate							
14	1.15	2					
26	2.30	2	−15	145.8	104.5	5.9	94.17
16	5.00	2					
Group IV, males, progesterone							
14	14	2	−3	148.5	110.0	5.2	94.16

[1] Electrolyte values expressed in millimols.
[2] Standard deviation.

(Selye, 1939, 1940; Shay, *et al.*, 1941; Ludden, *et al.*, 1941; Kochakian, 1941; Pfeiffer, *et al.*, 1940; Durlacher, *et al.*, 1842). It has been suggested that the tubular hypertrophy that follows the injection of desoxycorticosterone acetate was associated with the attempt of the tubules to re-absorb potassium in the presence of a deficiency of this cation (Durlacher, *et al.*, 1941). Since it was expected that the injection of the sex hormones over a period of weeks might lead to similar deficiencies of potassium in the organism, the wet and dry weights of the kidneys were determined and their histologic examination undertaken. It has also been shown that myocardial necrosis occurs in animals rendered deficient in potassium by the injection of desoxycorticosterone acetate or by feeding a diet low in potassium (Darrow and Miller, 1942). Hence, the hearts of the animals receiving repeated injections of the sex hormones were also studied.

METHODS AND MATERIAL

Adult albino rats weighing close to 300 gm. each were used throughout the present study. Injections of testosterone propionate, estradiol dipropionate, estradiol benzoate and progesterone were made in peanut or sesame oil every day or every other day.[1] The amount of hormone and the period of time over which the sex hormones were injected are shown in table 1. The methods of analysis for serum and muscle electrolyte were the same as those previously used in this laboratory (Harrison and Darrow, 1938). Both kidneys of each rat were weighed together for wet and dry weight. Sections of the kidney and the heart were stained with hematoxylin and eosin for

[1] The author is indebted to Dr. James B. Hamilton of the Department of Anatomy for the hormones used in the study of the rats in *Groups II, III and IV* of table 1. The hormones were prepared by Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

TABLE 1. (*continued*) EFFECT OF SEX HORMONES ON MUSCLE ELECTROLYTE AND KIDNEY WEIGHTS

TREATMENT			AVERAGE WEIGHT CHANGE %	MUSCLE ELECTROLYTES[1]/100 GM. FAT-FREE SOLIDS						KIDNEY WEIGHTS GM./KG.	
DAYS	TOTAL AMT. MG.	NO. OF RATS		Na	Cl	P	K	PROT. gm.	H_2O cc.	WET	DRY
				Group I, males, controls							
Control	12			8.8 ±1.3[2]	6.0 ±.63	32.4 ±1.22	48.6 ±.81	15.38 ±.48	340.1 ±5.0	7.62[3] ±0.41	1.77 ±0.09
				Group II, males, testosterone propionate							
14	14	2	+2	9.0	5.4	33.3	49.8	15.53	336.2	8.40	1.91
26	28	2									
				Group III, males, estradiol dipropionate							
14	1.15	2									
26	2.30	2	−15	10.3	5.5	32.2	46.6	15.47	332.3	7.20	1.52
16	5.00	2									
				Group IV, males, progesterone							
14	14	2	−3	9.3	4.9	32.7	45.5	15.43	316.0	7.27	1.72
(months)				*Group V,[4] males, testosterone propionate*							
6	57.5	1		7.7	6.1		46.1	15.00	337.0		
6[5]	57.5	1		8.2	5.5		43.4	14.76	329.0		
				Group VI, males, estradiol benzoate							
6	5.75	1		11.7	5.3		40.2	15.33	322.0		
6[5]	5.75	1		9.9	5.7		42.9	15.01	329.0		

[1] Electrolyte values expressed in millimols.
[2] Standard deviation.
[3] The data for the controls were taken from a study by Durlacher, Darrow and Winternitz (1942).
[4] The author is indebted to Dr. Philip LeCompte of the Department of Pathology for the injection and preparation of the 4 rats in *Group V*.
[5] Female animals.

microscopic study. The rats were fed on Purina dog chow until the day of autopsy. Water was permitted *ad libitum*. Rats in *Groups II, III* and *IV* of table 1 were weighed every other day throughout the period of injections.

RESULTS

In table 1 are shown the results of the analyses for serum and muscle electrolyte. In *Groups II, III*, and *IV* average results have been given. In *Group V* the results on individual rats are shown. Although the rats in *Group II* received large amounts of testosterone propionate over periods as long as a month, no significant change in serum or muscle electrolyte was found. However, the concentrations of potassium in the muscle of both the male and female rats in *Group V* receiving testosterone propionate over a period of 6 months were definitely low, being 46.1 and 43.5 mM per 100 gm. of fat-free solids, respectively. The concentration of potassium in the muscle of normal rats has been found to be 47 to 50 mM and figures below 44 mM have been observed only in animals receiving desoxycorticosterone acetate or diets low in potassium (Miller and Darrow, 1940).

The injection of the estrogenic hormones resulted in low-normal values for muscle potassium over periods as long as one month (*Group III*) and in abnormally low concentrations of muscle potassium when administered to a

male and female animal (*Group V*) over a period of 6 months (40.2 and 42.9 mM, respectively).

The supply of progesterone was limited, but in the amounts given it produced low-normal concentrations of potassium in the muscle of 2 rats in a period of 2 weeks (*Group IV*).

Significant replacement of intracellular potassium by sodium in the muscle was not observed except perhaps in the male rat in *Group V* receiving estradiol benzoate and in this animal the sodium concentration of 11.7 mM in the muscle was higher than expected in normal rats.

The increases in the wet and dry weights of the kidneys of the rats injected with testosterone propionate (*Group II*) were appreciable. The renal wet and dry weights of the rats receiving estradiol dipropionate were decreased. All renal weights were based on the body weight of the rat at the start of the experiment. The rats injected with estradiol benzoate all lost an average of 15 per cent of the original body weight during the periods of injection. Had the kidney weights been determined in relation to the final body weight of these rats, a 15 per cent increase in renal substance would have appeared to have occurred. No changes were seen in the histologic preparations from the kidneys or hearts of the rats in *Group II*, *III* and *IV*. None were made from the tissues of the rats in *Group V*.

DISCUSSION

On the basis of their ability to decrease the concentration of muscle potassium, the sex hormones can be arranged in a sequence of effectiveness with progesterone as the most potent, testosterone propionate as the least, and estradiol intermediate between the two. This is essentially in agreement with the observations of Thorn and Engel. The fact that these substances displace potassium from the muscle cell gives further justification for considering their action on electrolyte metabolism similar to that of desoxycorticosterone acetate but their degree of effectiveness is less since it is possible to demonstrate abnormal losses of potassium from the muscle of animals injected with desoxycorticosterone acetate for 9 days (Miller and Darrow, 1941) and comparable losses of potassium could be demonstrated for the sex hormones only after injections had been made over a period of 6 months. The failure to find any significant increase in the intracellular sodium of the muscle cell, except in one rat, was related to the relatively mild deficiencies of potassium encountered in the present study as compared to those observed in animals treated with desoxycorticosterone acetate (Miller and Darrow, 1941; Ferrebee, *et al.*, 1941; Darrow and Miller, 1942). These data emphasize that although the effect of the sex hormones was slight in promoting potassium excretion, this action was more pronounced than the sodium-retaining capacities. Increases in muscle water were not observed as the result of the injection of any of the sex hormones and, hence, it is unlikely that extracellular sodium was being retained by the organism.

It appears from this and other studies that the estrogens have 3 and testosterone propionate has at least 2, and possibly 3, effects on potassium metabolism. Their actions on potassium may best be considered in relation to nitrogen excretion. Potassium, together with nitrogen, may be retained following the injection of testosterone propionate over short periods of time, presumably in the building of cellular structures. A similar although less pronounced result has been described recently when estrogens are injected in small amounts (Knowlton, *et al.*, 1942). The second effect on potassium is an indirect one. Long has shown that the estrogens promote gluconeogenesis and presumably potassium accompanies the increased excretion of nitrogen in the urine as the result of the breakdown of cells (Long, 1942). The ratio of potassium to nitrogen in the urine has not, however, been determined in the experimental work on gluconeogenesis. Third, the present experiments indicate that the excretion of potassium may be relatively increased over that of nitrogen following the injection of testosterone propionate or estradiol. The only other explanation for the decrease in the concentration of muscle potassium observed here would presuppose a gain in body weight. If tissue mass were increased and if nitrogen were retained in greater amounts than potassium, the concentration of potassium in the tissues would be lowered. This possibility is excluded in the present experiments since no significant increments in body weight were observed and an actual loss of considerable weight occurrred in rats injected with estradiol dipropionate. It is reasonable to suppose that nitrogen may be retained in greater amounts than potassium, although no direct observations have been made on this point. It has been recently shown, however, that extremely low concentrations of potassium may be demonstrated in the serum of children receiving methyl testosterone and testosterone propionate at a time when the tissue mass, total tissue potassium and tissue nitrogen were being increased (Butler, *et al.*, 1942). From a large experience with analyses of serum and muscle potassium concentrations in animals, low concentrations of the cation in the serum have always been associated with abnormally low concentrations of potassium in the muscle (Miller and Darrow, 1940, 1941; Darrow and Miller, 1942). It may be presumed that in the case of the children nitrogen and potassium were being retained so that the concentration of potassium was less than normal. In the experiments of Thorn and Engel (1938, *b*) and Kenyon, *et al* (1938) while potassium was being retained with nitrogen, it was in amounts such as to keep the potassium in the tissues at a normal concentration or at a concentration higher than normal.

SUMMARY

Concentrations of potassium were found to be abnormally low in the muscle of rats injected with estradiol benzoate and testosterone propionate over a period of several months. On the basis of their effectiveness in decreasing the concentration of potassium in the muscle, progesterone, estradiol and testosterone may be ranked in descending order.

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NOTES AND COMMENTS

EFFECT OF CHRONIC INSULIN[1] INJECTIONS ON REPRODUCTION IN WHITE RATS

THE PREPARATION of insulin in a form which was safe for therapeutic use has been one of the greater discoveries in medical science during this century. The fact that it is now being used for conditions other than that of diabetes mellitus has made it even more important and of greater interest. While a great deal of work of an experimental nature has been done in determining the effect or effects of this hormone on various vital body processes, there are few reports of the results of sub-shock doses of insulin and of insulin administration over long periods of time. This study was undertaken in an effort to study these two problems experimentally in several successive generations of white rats. The effect of insulin on growth was reported by Ferrill (1941) and on histologic and gross structure by Low and Ferrill (1941).

Procedure. Rats of a known strain and age were obtained to begin the colony and first litters born to these animals were designated as generation 1. The number of animals per litter, the sex and weight of each, were recorded on the day of birth and then the litter reduced to six in number. At the age of 24 days all animals were weaned and intraperitoneal insulin injections were begun and made daily except Sunday. It was found that most of the animals under the experimental conditions could withstand 20 units of insulin per kg. of body weight per day, in a single dose (morning), or 40 units per kg. of body weight per day, if given two doses, one in the morning and one in late afternoon. Some animals with either procedure went into shock and a few were lost. Food (Purina chow) and water were available at all times and green vegetables were fed twice per week.

The injection procedures were maintained from the age of 24 days until the animal reproduced and the litter was weaned. The animals were permitted to breed as early as possible, males being kept with the females until the latter were known to be pregnant. The same males were not kept indefinitely with the same females, but were changed every few days because it has been our experience that occasionally a given pair of rats did not reproduce while either the male or female when placed with another mate would breed and cast perfectly normal litters.

The control animals were of two groups one of which was given intraperitoneal injections of 0.9 per cent salt solution in amounts comparable in volume to the insulin given for that weight; the other group had no injections. This report deals with only the females of the various generations and takes into consideration the age of the animals at the time the first litter was born, the number in each litter, the number of males and females in each litter, and the number of animals dead at birth or dying during first day. The data from the experimental animals have been combined for study since there was no detectable difference between those receiving the 20 units and those the 40 units. Likewise, in table 1, the data for the controls receiving saline and the untreated controls have been grouped together for the same reason. The total number of animals studied was 300, 156 of which were experimental animals and 144 controls.

Results. In table 1, are recorded the number of animals, experimental and control, for each generation through the fifth, and other pertinent data.

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[1] Acknowledgement is made of the generous supplies of insulin furnished by Eli Lilly & Co., Indianapolis, Ind.

In generations 3 and 5 the average age of the mothers when the first litter was cast appears to be significantly greater than in the other 3 generations, but when control and experimental animals for these generations are compared, this is not the case. These deviations may possibly be explained on the basis of seasonal variation, since these two generations were breeding during the fall months of the year. Furthermore, even these ages do not fall outside the normal variations as found by Long and Evans (1922). The average they reported was somewhat lower, but the range was from 45 to 147 days.

TABLE 1. EFFECT OF PROLONGED TREATMENT WITH SUB-SHOCK DOSES OF INSULIN ON REPRODUCTION IN FEMALE WHITE RATS

GENERATION NO.	NO. OF ANIMALS	AV. AGE WHEN 1ST LITTER BORN	AV. NO. IN LITTER	AV. NO. MALES	AV. NO. FEMALES	DEAD
		days				
1, Experimental	18	88.3	9.7	4.4	5.2	0.41
1, Control	18	90.7	9.7	4.5	5.2	0.83
2, Experimental	25	89.7	8.8	4.6	4.2	0.37
2, Control	23	92.8	8.8	4.2	4.5	0.36
3, Experimental	35	147.0	8.2	4.0	4.2	0.25
3, Control	31	121.6	8.8	4.2	4.6	0.82
4, Experimental	40	99.6	7.4	3.6	3.7	0.66
4, Control	45	96.7	7.3	3.6	3.6	0.44
5, Experimental	38	114.6	9.0	3.5	5.0	0.57
5, Control	27	137.4	5.6	1.6	3.1	0.95

There was a slight tendency for the size of the litter to decrease (about 1) from generation to generation. The proportion of males to females born was consistent except in the fifth generation, in which there was a preponderance of females.

Summary and Conclusions. The amounts of insulin used in the experimental rats were greater in proportion to body weight than have been used therapeutically over long periods of time in man. From the results of this study it can be concluded that insulin given in amounts of 20 to 40 units per kg. of body weight per day has no significant effect on reproduction in the white rat in one generation or in 5 succeeding generations.

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NEW BOOKS

PEDIATRIC GYNECOLOGY. Goodrich C. Schauffler, Assistant Clinical Professor of Obstetrics and Gynecology, University of Oregon Medical School; Visiting Gynecological Surgeon and Obstetrician, Multnomah Hospital, Portland, Ore. $5.00. 384 pages; 2 tables, 66 figures. Chicago, Ill. The Year Book Publishers, Inc., 1942.

Those practitioners of medicine who are called upon to treat gynecologic disorders in infants and children have often encountered difficulty in obtaining adequate advice and therapy for their patients. Most pediatricians have had little practical experience in dealing with these problems, and the expert gynecologist has usually paid little attention to diseases appearing in pre-adolescent years. A considerable gap has therefore existed in medical practice, and to bridge this neglected field between medicine, pediatrics and gynecology Schauffler has made a notable contribution by setting forth in a monograph the various genito-urinary problems which might be encountered in girls and female infants. Here is a single volume to which the doctor can turn for a consideration of almost any pathologic condition of the urinary system or female genital tract in the early portion of life.

The subject matter has been systematically presented. Points of history-taking, physical examination, special diagnostic procedures and therapy are individually treated for each of the various disorders which are listed. The text has a lucid style. Adequate drawings, photographs, sketches, and roentgenograms are provided where needed, except for illustration of surgical procedures. The scope of the work is broad. It summarizes the general reactions of the child as compared to those of the adult; it points out the anatomic differences between the child and the adult; it supplies treatises on vaginal infection, disorders of the menarche, breast lesions, abnormalities and tumors of the urinary tract, as well as malformations and some other conditions of the colon and rectum. The author has set forth in didactic fashion what he believes to be the best method of treatment for each disease which is discussed.

While the limited number of references to the literature support the statements made in the text, they do not provide a sufficiently wide source for those readers who might want to pursue their studies further and obtain divergent views on some matters which are highly controversial. Hence, for practical purposes, the bibliography is too limited. It appears that undue emphasis and allotment of space are given to some subjects, such as vaginitis and conditions of the external genitalia, while there is a slighting of general surgical considerations and congenital malformations of the genito-urinary organs. On the whole, the book will have considerable usefulness for the general practitioner or pediatrician, since greatest stress has been laid on those therapeutic procedures which come under the general heading of office treatments. Unfortunately, the surgeon will find little aid, because operative techniques have been given very little attention in a book in which they could well take a prominent place. Likewise, the endocrinologist will be disappointed by the complete disregard of genital abnormalities caused by disturbed endocrine balance or by neoplasms of the various glands of internal secretion.

ROBERT E. GROSS

THE ADRENAL CORTEX IN ADAPTATION TO ALTITUDE, CLIMATE AND CANCER. Edward S Sundstroem and George Michaels, Department of Biochemistry, University of California, Berkeley, Calif. Paper. $4.00, viii + 410 pages; 125 text figures. Berkeley and Los Angeles, Calif. University of California Press, 1942.

This curious document poses a difficult problem to the conscientious reviewer largely because what might be called the tone of the volume differs materially from that usually found in monographs of similar purpose. At the very outset, for instance, one is informed that the junior author assumes "major responsibility for the accuracy of the observation records," while the senior author "is responsible for the accuracy of most final results and for the text and interpretations." Whatever may have been the good intentions of this disclaimer, the reader is inescapably left with the following impressions: *a*) that neither party fully concurred in the doings of the other, and *b*) that the final results were not importantly conditioned by the experimental observations.

The final bibliography closes with a footnote to the effect that "No attempt has been made to extend to 1942 the bibliography . . . Such an attempt would have compelled us to rewrite several parts" (most of which were completed between 1935 and 1937). In view of the rapid progress made in adrenal physiology since these dates, it almost appears as if the authors are submitting this handsomely printed quarto strictly as a collector's item. Perhaps a sly dig is directed at the publishers for having brooded over the manuscript for an admitted two of the four years prior to publication. Another explanation for the delay is found, however, in the hypothesis that the entire manuscript was lost for four years. This proposition rests upon the repeated precedents cited in the text in which the protocols of entire experimental series and in one instance the microscopic slides of an important tumor were mislaid before they had been studied and interpreted. In another place we are actually asked to ignore a published figure on the grounds that other experiments of the same sort, the results of which are unavailable, gave different and for their theory more satisfactory results.

The book is full of apologies of all sorts, not only for losing so many protocols, but for not having set up many of the experiments differently, for omitting certain crucial experiments which suggested themselves, and especially for failures to give adequate statistical treatment to the material. The latter omission seems particularly unfortunate since the authors state categorically that the method of experimentation adopted "made the evaluation of many of the data a statistical procedure." The reasons advanced for omitting such matters as standard error and the like are not moreover reassuring: *a*) space economy (p. 28); *b*) "it would have been very laborious"; and *c*) "we were interested in qualitative rather than quantitative changes" (p. 289).

Perhaps enough has been said to illustrate what was referred to above as the 'tone' of the volume. One more example may appropriately serve to close this subject: " . . . it was not realized immediately that mortality rates (and also changes in body and organ weights and body temperature) may serve as convenient measures of the relative tolerance of the animals to various reductions of pressure. More such data would have been available had we begun earlier to keep careful records of dates of death" etc.

The major portion of the experimental work falls into two main divisions: 1, the responses of normal rats to atmospheric rarefaction; and, 2, the effect of the latter upon *in vivo* growth of animal tumors, with some supplementary work on infectious diseases.

Section 1 is divided into two subsections. The first is an elaborate collection of data on changes in respiration, metabolism, and blood and tissue elements under a very wide range of pressures. The methods and especially the apparatus developed for keeping various species of animals under various levels of barometric pressure for long uninterrupted periods of time struck the reviewer as very ingenious in many points, although obviously too expensive and elaborate for all but a few laboratories. These experiments seem for the most part to have been intelligently and thoroughly carried out, and should constitute a definite contribution to our knowledge. Only occasionally is the technical picture marred by such matters as a normal potassium level some 150% of normal. One can only regret that much of this material remained unpublished during the past decade when so many other workers have been con-

cerned with high altitude problems. The earlier submission of these data to the scientific public might also have resulted in a critical analysis, useful not only to the authors in many of their subsequent speculations, but to others concerned in the present pressing problem of high altitude flight.

The second subsection deals with general theories of mountain sickness and adjustment to high altitudes, and specifically with the rôle of the adrenal cortex. The introductory theoretical review is somewhat loosely done, the 'acapnic' theory being dismissed without discussion as obsolete, a decision which is unfortunate in view of recent work. Furthermore, the anoxic theory is pitted against the adrenal exhaustion theory, in spite of the fact that the function of the cortex is thought by the authors to be primarily one of enabling the tissues to get along on low oxygen tensions. Incidentally several of the theoretical discussions gave the reviewer an impression of elaborate treatments of the general problem represented by the simple case—if a man has difficulty reaching an object, is it because his arms are too short or because the object is too far away?

In an earlier section there is a prolonged consideration of the weight loss experienced by all the animals under low pressure. Although this was shown to be approximately equal to that shown by controls fed the same amount of food as the low pressure animals ate spontaneously, great obscurities arise in the discussion which closes: "Were the alterations of these factors (weight loss, under-nutrition or deficient appetite) the only unfavorable findings it would be difficult to hold the low pressure as such responsible for them. However, our simultaneous discovery of several other functional alterations . . . makes the conclusion almost unavoidable that atmospheric rarefaction was the main, if not the only, cause of the extreme loss of weight in these rats. It seems indeed doubtful whether a lessened food intake had any direct connection. . . . A large part of this wasting might have taken place even with forced feeding." It seems a pity, since an operational answer to at least part of this bizarre question was suggested, that the appropriate experiments were not carried out. The reviewer dwelt for some time upon these and related sentences, but still finds the implications for the law of conservation of mass too intriguing to be so lightly neglected.

In spite of the looseness of the theoretical treatment, it appears that the experimental results establish the main point, *i.e.*, that the adrenal cortex protects against the effects of low pressure. The failure of adrenalectomized animals to withstand lower pressure was clearly demonstrated and the amount of cortical extract needed to protect these and normal animals was found to vary directly with the reduction of pressure. Several subsidiary experiments add to this convincing chain of evidence and the importance of the point goes a long way in mitigating impatience with other features of the treatise.

The section on tumors is more difficult to defend. In view of the immense complexities and numerous pitfalls which beset the field of tumor growth, skillful flanking procedures appear at present to be the only method of attack. The authors of the present work have, however, boldly attacked a wide front running from the high ground of transplantable cancers down through a maze of spontaneous growths to end finally in the confusing area of the Rous Chicken Sarcoma. In view of the difficulties of the terrain, too much reliance appears to have been placed on untrained troops but recently drafted from the civilian life of endocrinology.

Even if one accepts the evidence that exposure to low pressure caused regression of advanced tumors, it is difficult to agree with the thesis that it was due specifically to the high levels of adrenal cortical hormone presumably induced by the low pressure. Nowhere is it convincingly demonstrated, for instance, that the maximum regression coincides in time with maxima of adrenal secretion; in fact, in some of the experiments regression proceeded in animals exposed for very long periods of time to pressure so low that, as demonstrated in the earlier section, adrenal exhaustion may have supervened. Support for the latter view is found in the fact that several of these animals died with the tumor still regressing.

The notorious frequency with which tumors fail to grow or actually regress in cachectic animals is recognized by the authors, but somewhat cavalierly excluded as an unimportant factor in their experiments. The failure of administered adrenal cortex extract to bring about regression in tumors also constitutes a serious drawback to the hypothesis. An effort to dis-

count this failure on the grounds of inadequate dosage may reasonably be doubted on the basis of evidence produced elsewhere in the book. On p. 156 it is shown that enough cortical extract can be given adrenalectomized rats to enable them to live for ten days at a pressure of 360 mm. Since some normal rats were unable to live at this pressure, and lower pressures increased the mortality rate sharply (p. 36), it is reasonable to suppose that the normal rat adrenal is barely capable of secreting a dose of cortical hormone adequate for maintenance at this pressure, which, as it happens, is also optimal pressure for tumor treatment (p. 216). Since such doses of cortical extract are readily given artificially, the finding (p. 319) that 'large doses' were powerless to affect tumor growth leads the reviewer at least to feel that if low pressure influences tumors it does so by means other than the intermediation of the adrenal cortex.

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